METHODS AND NUCLEIC ACIDS FOR ANALYSES OF COLORECTAL CELL PROLIFERATIVE DISORDERS

FIELD OF THE INVENTION

The present invention relates to genomic DNA sequences that exhibit altered CpG methylation patterns in disease states relative to normal. Particular embodiments provide methods, nucleic acids, nucleic acid arrays and kits useful for detecting, or for detecting and differentiating between or among colorectal cell proliferative disorders.

SEQUENCE LISTING

A Sequence Listing, pursuant to 37 C.F.R. § 1.52(e)(5), has been provided on compact disc (1 of 1) as a 3.048 MB file, entitled 47675-47.txt, and which is incorporated by reference herein in its entirety.

BACKGROUND

The etiology of pathogenic states is known to involve modified methylation patterns of individual genes or of the genome. 5-methylcytosine, in the context of CpG dinucleotide sequences, is the most frequent covalently modified base in the DNA of eukaryotic cells, and plays a role in the regulation of transcription, genetic imprinting, and tumorigenesis. The identification and quantification of 5-methylcytosine sites in a specific specimen, or between or among a plurality of specimens, is thus of considerable interest, not only in research, but particularly for the molecular diagnoses of various diseases.

Correlation of aberrant DNA methylation with cancer. Aberrant DNA methylation within CpG 'islands' is characterized by *hyper-* or *hypomethylation* of CpG dinucleotide sequences leading to abrogation or overexpression of a broad spectrum of genes, and is among the earliest and most common alterations found in, and correlated with human malignancies. Additionally, abnormal methylation has been shown to occur in CpG-rich regulatory elements in intronic and coding parts of genes for certain tumors. In colon cancer, for example, aberrant DNA methylation constitutes one of the most prominent alterations and inactivates

many tumor suppressor genes such as p14ARF, p16INK4a, THBS1, MINT2, and MINT31 and DNA mismatch repair genes such as hMLH1.

In contrast to the specific hypermethylation of tumor suppressor genes, an overall hypomethylation of DNA can be observed in tumor cells. This decrease in global methylation can be detected early, far before the development of frank tumor formation. A correlation between hypomethylation and increased gene expression has been determined for many oncogenes.

Colorectal Cancer. Colorectal cancer is the fourth leading cause of cancer mortality in men and women, although ranking third in frequency in men and second in women. The 5-year survival rate is 61% over all stages with early detection being a prerequisite for curative therapy of the disease. Up to 95% of all colorectal cancers are adenocarcinomas of varying differentiation grades.

Sporadic colon cancer develops in a multistep process starting with the pathologic transformation of normal colonic epithelium to an adenoma which consecutively progresses to invasive cancer. The progression rate of benign colonic adenomas depends strongly on their histologic appearance: whereas tubular-type adenomas tend to progress to malignant tumors very rarely, villous adenomas, particularly if larger than 2 cm in diameter, have a significant malignant potential.

During progression from benign proliferative lesions to malignant neoplasms several genetic and epigenetic alterations occur. Somatic mutation of the APC gene seems to be one of the earliest events in 75 to 80% of colorectal adenomas and carcinomas. Activation of K-RAS is thought to be a critical step in the progression towards a malignant phenotype. Consecutively, mutations in other oncogenes as well as alterations leading to inactivation of tumor suppressor genes accumulate.

In the molecular evolution of colorectal cancer, DNA methylation errors have been suggested to play two distinct roles. In normal colonic mucosa cells, methylation errors accumulate as a function of age or as time-dependent events predisposing these cells to neoplastic transformation. For example, hypermethylation of several loci could be shown to be already present in adenomas, particularly in the tubulovillous and villous subtype. At later

stages, increased DNA methylation of CpG islands plays an important role in a subset of tumors affected by the so called CpG island methylator phenotype (CIMP). Most CIMP+ tumors, which constitute about 15% of all sporadic colorectal cancers, are characterized by microsatellite instability (MIN) due to hypermethylation of the hMLH1 promoter and other DNA mismatch repair genes. By contrast, CIMP- colon cancers evolve along a more classic genetic instability pathway (CIN), with a high rate of p53 mutations and chromosomal changes.

However, the molecular subtypes do not only show varying frequencies regarding molecular alterations. According to the presence of either micro satellite instability or chromosomal aberrations, colon cancer can be subclassified into two classes, which also exhibit significant clinical differences. Almost all MIN tumors originate in the proximal colon (ascending and transversum), whereas 70% of CIN tumors are located in the distal colon and rectum. This has been attributed to the varying prevalence of different carcinogens in different sections of the colon. Methylating carcinogens, which constitute the prevailing carcinogen in the proximal colon have been suggested to play a role in the pathogenesis of MIN cancers, whereas CIN tumors are thought to be more frequently caused by adduct-forming carcinogens, which occur more frequently in distal parts of the colon and rectum. Moreover, MIN tumors have a better prognosis than do tumors with a CIN phenotype and respond better to adjuvant chemotherapy.

Incidence and mortality rates for this disease increase greatly with age, particularly after the age of 60. Stage of disease at diagnosis also affects overall survival rates. Patients having lesions confined to the colonic wall have a high probability of surviving 5 or more years while patients with metastatic disease have a very low probability of survival. It is thought that most colorectal cancers develop over a course of 5-10 years from a precursor lesion called an adenomatous polyp. The potential of these lesions to result in adenocarcinoma has been shown to increase with both polyp size and degree of dysplasia. Because of the slow progression of this disease, early detection through routine screening can result in significant improvement of survival rates. Several randomized trials over the last 20 years have shown that screening test can reduce mortality over 30%, even though the tests

used were not highly sensitive. The current guidelines for colorectal screening according to the American Cancer Society utilizes one of five different options for screening in average risk individuals 50 years of age or older. These options include 1) fecal occult blood test (FOBT) annually, 2) flexible sigmoidoscopy every five years, 3) annual FPBT plus flexible sigmoidoscopy every five years, 4) double contrast barium enema (DCBE) every five years or 5) colonoscopy every ten years. Even though these testing procedures are well accepted by the medical community, the implementation of widespread screening for colorectal cancer has not been realized. Patient compliance is a major factor for limited use due to the discomfort or inconvenience associated with the procedures. FOBT testing, although a non-invasive procedure, requires dietary and other restrictions 3-5 days prior to testing. Sensitivity levels for this test are also very low for colorectal adenocarcinoma with wide variability depending on the trial. Sensitivity measurements for detection of adenomas is even less since most adenomas do not bleed. In contrast, sensitivity for more invasive procedures such as sigmoidoscopy and colonoscopy are quite high because of direct visualization of the lumen of the colon. No randomized trials have evaluated the efficacy of these techniques, however, using data from case-control studies and data from the National Polyp Study (U.S.) it has been shown that removal of adenomatous polyps results in a 76-90% reduction in CRC incidence. Sigmoidoscopy has the limitation of only visualizing the left side of the colon leaving lesions in the right colon undetected. Both scoping procedures are expensive, require cathartic preparation and have increased risk of morbidity and mortality. Improved tests with increased sensitivity, specificity, ease of use and decreased costs are clearly needed before general widespread screening for colorectal cancer becomes routine.

Molecular disease markers offer several advantages over other types of markers, one advantage being that even samples of very small sizes and/or samples whose tissue architecture has not been maintained can be analyzed quite efficiently. Within the last decade a number of genes have been shown to be differentially expressed between normal and colon carcinomas. However, no single or combination of marker has been shown to be sufficient for the diagnosis of colon carcinomas. High-dimensional mRNA based approaches have recently been shown to be able to provide a better means to distinguish between different tumor types

and benign and malignant lesions. However its application as a routine diagnostic tool in a clinical environment is impeded by the extreme instability of mRNA, the rapidly occurring expression changes following certain triggers (*e.g.*, sample collection), and, most importantly, the large amount of mRNA needed for analysis (Lipshutz, R. J. et al., Nature Genetics 21:20-24, 1999; Bowtell, D. D. L. Nature genetics suppl. 21:25-32, 1999), which often cannot be obtained from a routine biopsy.

There is a need in the art for a sensitive diagnostic or prognostic assay for colon cell proliferative disorders that is based, at least in part, on detection of differential methylation of CpG dinucleotide sequences, and that has a diagnostic or prognostic accuracy of greater than about 80%, preferably greater than about 85% or about 90%, more preferably greater than about 95%, and most preferably greater than about 98%.

SUMMARY OF THE INVENTION

The present invention provides novel methods for detecting or distinguishing between colorectal cell proliferative disorders. Said method is most preferably utilised for detecting or detecting and distinguishing between one or more of the following: colorectal carcinoma, colon adenoma, inflammatory colon tissue, grade 2 dysplasia colon adenomas less than 1 cm, grade 3 dysplasia colon adenomas larger than 1 cm, normal colon tissue, non-colon normal tissue, body fluids and non-colon cancer tissue. The invention provides a method for the analysis of biological samples for features associated with the development of colon cell proliferative disorders, the method characterised in that at least one nucleic acid, or a fragment thereof, from the group consisting of SEQ ID NO:1 to SEQ ID NO:535 is/are contacted with a reagent or series of reagents capable of distinguishing between methylated and non methylated CpG dinucleotides within the genomic sequence, or sequences of interest.

The present invention provides a method for ascertaining genetic and/or epigenetic parameters of genomic DNA. The method has utility for the improved diagnosis, treatment and monitoring of colon cell proliferative disorders, more specifically by enabling the improved identification of and differentiation between subclasses of said disorder and the genetic predisposition to said disorders. The invention presents improvements over the state

of the art in that it enables a highly specific classification of colon cell proliferative disorders, thereby allowing for improved and informed treatment of patients.

Preferably, the source of the test sample is selected from the group consisting of cells or cell lines, histological slides, biopsies, paraffin-embedded tissue, bodily fluids, ejaculate, urine, blood, and combinations thereof. Preferably, the source is biopsies, bodily fluids, ejaculate, urine, or blood.

Specifically, the present invention provides a method for detecting colon cell proliferative disorders, comprising: obtaining a biological sample comprising genomic nucleic acid(s); contacting the nucleic acid(s), or a fragment thereof, with one reagent or a plurality of reagents sufficient for distinguishing between methylated and non methylated CpG dinucleotide sequences within a target sequence of the subject nucleic acid, wherein the target sequence comprises, or hybridizes under stringent conditions to, a sequence comprising at least 16 contiguous nucleotides of SEQ ID NO:1 to 535, said contiguous nucleotides comprising at least one CpG dinucleotide sequence; and determining, based at least in part on said distinguishing, the methylation state of at least one target CpG dinucleotide sequence, or an average, or a value reflecting an average methylation state of a plurality of target CpG dinucleotide sequences. Preferably, distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence comprises methylation state-dependent conversion or non-conversion of at least one such CpG dinucleotide sequence to the corresponding converted or non-converted dinucleotide sequence within a sequence selected from the group consisting of SEQ ID NO:304 to SEQ ID NO:535, and contiguous regions thereof corresponding to the target sequence.

Additional embodiments provide a method for the detection of colon cell proliferative disorders, comprising: obtaining a biological sample having subject genomic DNA; extracting the genomic DNA; treating the genomic DNA, or a fragment thereof, with one or more reagents to convert 5-position unmethylated cytosine bases to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties; contacting the treated genomic DNA, or the treated fragment thereof, with an amplification enzyme and at least two primers comprising, in each case a contiguous sequence at least 9 nucleotides in length that is

complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting SEQ ID NO:304 to SEQ ID NO:535, and complements thereof, wherein the treated DNA or the fragment thereof is either amplified to produce an amplificate, or is not amplified; and determining, based on a presence or absence of, or on a property of said amplificate, the methylation state of at least one CpG dinucleotide sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:58, or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotide sequences thereof. Preferably, at least one such hybridizing nucleic acid molecule or peptide nucleic acid molecule is bound to a solid phase. Preferably, determining comprises use of at least two methods selected from the group consisting of: hybridizing at least one nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NO:304 to SEQ ID NO:535, and complements thereof; hybridizing at least one nucleic acid molecule, bound to a solid phase, comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NO:304 to SEQ ID NO:535, and complements thereof; hybridizing at least one nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NO:304 to SEQ ID NO:535, and complements thereof, and extending at least one such hybridized nucleic acid molecule by at least one nucleotide base; and sequencing of the amplificate.

Further embodiments provide a method for the analysis of colon cell proliferative disorders, comprising: obtaining a biological sample having subject genomic DNA; extracting the genomic DNA; contacting the genomic DNA, or a fragment thereof, comprising one or more sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:58 or a sequence that hybridizes under stringent conditions thereto, with one or more methylation-sensitive restriction enzymes, wherein the genomic DNA is either digested thereby to produce digestion fragments, or is not digested thereby; and determining, based on a presence or

absence of, or on property of at least one such fragment, the methylation state of at least one CpG dinucleotide sequence of one or more sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:58, or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotide sequences thereof. Preferably, the digested or undigested genomic DNA is amplified prior to said determining.

Additional embodiments provide novel genomic and chemically modified nucleic acid sequences, as well as oligonucleotides and/or PNA-oligomers for analysis of cytosine methylation patterns within sequences from the group consisting of SEQ ID NO:1 to SEQ ID NO:58.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1, 5, 9, 13, 16, 20, 24, 28 and 32 show ranked matrices of data obtained according to EXAMPLES 1 and 2, and according to CpG methylation differences between the two classes of tissues, using a suitable algorithm. The figures are shown in greyscale, wherein the most significant CpG positions are at the bottom of the matrix with significance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in grey, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. The p-values for the individual CpG positions are shown on the right side. The p-values are the probabilities that the observed distribution occurred by chance in the data set.

Figures 2, 6, 10, 17, 21, 25, 29 and 33 show the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figures 3, 7, 11, 14, 18, 22, 26, 30 and 34 show ranked matrices of data, obtained according to EXAMPLES 1 and 2, of the accuracy of the genewise linear support vector machine cross validations between the two classes of tissues, for the best performing markers. The figures are shown in greyscale, wherein the most significant CpG positions are at the

bottom of the matrix with significance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in grey, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. Accuracy values for each individual genomic region of interest are shown on the right side.

Figures 4, 8, 12, 15, 18, 23, 27, 31 and 35 show the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification. The accuracy of each genomic region is represented as black squares, the specificity as unfilled diamonds, and the sensitivity as unfilled squares. The accuracy as measured on the X-axis shows the fraction of correctly classified samples.

Figure 36 shows the level of methylation determined by different MSP MethyLight assays and HeavyMethyl MethyLight assays. The Y-axis shows the degree of methylation. Tumor samples are represented by white points, and normal colon tissue samples by black points. A significantly higher degree of methylation was observed in tumor samples than in healthy tissue samples.

Figure 37 shows the Receiver Operating Characteristic curve (ROC curve) of the SEQ ID NO:35 -MSP-Methyl-Light-Assay for adenocarcinomas according to EXAMPLE 2. The AUC for the MSP-Methyl-Light-Assay is: 0.94.

Figure 38 shows the Receiver Operating Characteristic curve (ROC curve) of the SEQ ID NO:35 -HM-Methyl-Light-Assay for Adenocarcinoma according to EXAMPLE 3. The AUC for the HM-Methyl-Light-Assay is: 0.91.

Figure 39 shows the level of methylation determined by a SEQ ID NO:35 - HeavyMethyl MethyLight™ assay according to EXAMPLE 3, testing an additional set of colon samples (25 adenocarcinoma, 33 normals, and 13 adenomas). The Y-axis shows the degree of methylation within the region of the SEQ ID NO:35 gene investigated. Adenocarcinoma samples are represented by white squares, and normal colon tissue samples by black diamonds. A significantly higher degree of methylation was observed in tumor

samples than in healthy tissue samples. The level of significance as measured using a t-test was 0.00424.

Figure 40 shows the Receiver Operating Characteristic curve (ROC curve) of the SEQ ID NO:35 -HM-Methyl-Light-Assay for Adenocarcinoma and Adenoma according to EXAMPLE 3 (additional sets of samples). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test. The AUC for the HM-Methyl-Light-Assay is 0.81.

Figure 41 shows the Receiver Operating Characteristic curve (ROC curve) of the SEQ ID NO:35 -HM-Methyl-Light-Assay for Adenocarcinoma only according to EXAMPLE 2 (additional sets of samples). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test. The AUC for the HM-Methyl-Light-Assay is: 0.844.

Figure 42 shows the Receiver Operating Characteristic curve (ROC curve) of the SEQ ID NO:35 -HM-Methyl-Light-Assay for Adenenomas according to EXAMPLE 3 (additional sets of samples). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test. The AUC for the HM-Methyl-Light-Assay is: 0.748.

Figure 43 shows the level of methylation in different tumor and healthy tissues determined by a SEQ ID NO 35 -HeavyMethyl MethyLight™ assay according to example 4. The Y-axis shows the degree of methylation within the region of the SEQ ID NO:35 gene investigated. Besides the colon cancer samples only one of the two breast cancer tissues were methylated.

Figure 44 shows the level of methylation in different breast cancer tissues determined by a SEQ ID NO:35 -HeavyMethyl MethyLight™ assay according to EXAMPLE 4. Only one was methylated.

Figure 45 shows the level of methylation in serum samples determined by a SEQ ID NO:35 -HeavyMethyl MethyLight ™ assay according to EXAMPLE 4. The Y-axis shows the degree of methylation within the region of the SEQ ID NO:35 gene investigated.

Figure 46 shows the ROC curve of the SEQ ID NO:34 -MSP-Methyl-Light ™-Assay according to EXAMPLE 9. The AUC is: 0.84.

Figure 47 shows the ROC curve of the SEQ ID NO:29 -MSP-Methyl-Light ™-Assay according to EXAMPLE 10. The AUC is: 0.80.

Figure 48 shows the regression plot of the percentage methylation within SEQ ID NO:35 calculated in each sample using the MSP and HeavyMethyl ™ variants of the MethyLight ™ assay.

Figure 49 shows the ROC curve of the SEQ ID NO:29 -MSP-Methyl-Light ™-Assay according to EXAMPLE 8 (first sample set). The AUC is: 0.93.

Figure 50 shows the ROC curve of the SEQ ID NO:29 -MSP-Methyl-Light ™-Assay according to EXAMPLE 8 (second sample set). The AUC is: 1.

Figure 51 shows the ROC curve of the SEQ ID NO:39 -MSP-Methyl-Light ™-Assay according to EXAMPLE 9. The AUC is: 0.94.

DETAILED DESCRIPTION OF THE INVENTION

Definitions:

The term "Observed/Expected Ratio" ("O/E Ratio") refers to the frequency of CpG dinucleotides within a particular DNA sequence, and corresponds to the [number of CpG sites / (number of C bases × number of G bases)] × band length for each fragment.

The term "CpG island" refers to a contiguous region of genomic DNA that satisfies the criteria of (1) having a frequency of CpG dinucleotides corresponding to an "Observed/Expected Ratio" >0.6, and (2) having a "GC Content" >0.5. CpG islands are typically, but not always, between about 0.2 to about 1 kb, or to about 2kb in length.

The term "methylation state" or "methylation status" refers to the presence or absence of 5-methylcytosine ("5-mCyt") at one or a plurality of CpG dinucleotides within a DNA sequence. Methylation states at one or more particular palindromic CpG methylation sites (each having two CpG CpG dinucleotide sequences) within a DNA sequence include "unmethylated," "fully-methylated" and "hemi-methylated."

The term "hemi-methylation" or "hemimethylation" refers to the methylation state of a palindromic CpG methylation site, where only a single cytosine in one of the two CpG dinucleotide sequences of the palindromic CpG methylation site is methylated (*e.g.*, 5'-CC^{M}GG-3' (top strand): 3'-GGCC-5' (bottom strand)).

The term “hypermethylation” refers to the average methylation state corresponding to an *increased* presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample.

The term “hypomethylation” refers to the average methylation state corresponding to a *decreased* presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample.

The term “microarray” refers broadly to both “DNA microarrays,” and ‘DNA chip(s),’ as recognized in the art, encompasses all art-recognized solid supports, and encompasses all methods for affixing nucleic acid molecules thereto or synthesis of nucleic acids thereon.

“Genetic parameters” are mutations and polymorphisms of genes and sequences further required for their regulation. To be designated as mutations are, in particular, insertions, deletions, point mutations, inversions and polymorphisms and, particularly preferred, SNPs (single nucleotide polymorphisms).

“Epigenetic parameters” are, in particular, cytosine methylations. Further epigenetic parameters include, for example, the acetylation of histones which, however, cannot be directly analyzed using the described method but which, in turn, correlate with the DNA methylation.

The term “bisulfite reagent” refers to a reagent comprising bisulfite, disulfite, hydrogen sulfite or combinations thereof, useful as disclosed herein to distinguish between methylated and unmethylated CpG dinucleotide sequences.

The term “Methylation assay” refers to any assay for determining the methylation state of one or more CpG dinucleotide sequences within a sequence of DNA.

The term “MS.AP-PCR” (Methylation-Sensitive Arbitrarily-Primed Polymerase Chain Reaction) refers to the art-recognized technology that allows for a global scan of the genome using CG-rich primers to focus on the regions most likely to contain CpG dinucleotides, and described by Gonzalgo et al., *Cancer Research* 57:594-599, 1997.

The term "MethyLight™" refers to the art-recognized fluorescence-based real-time PCR technique described by Eads et al., *Cancer Res.* 59:2302-2306, 1999.

The term "HeavyMethyl™" assay, in the embodiment thereof implemented herein, refers to a HeavyMethyl™ MethylLight ™ assay, which is a variation of the MethylLight ™ assay, wherein the MethylLight ™ assay is combined with methylation specific *blocking* probes covering CpG positions between the amplification primers.

The term "Ms-SNuPE" (Methylation-sensitive Single Nucleotide Primer Extension) refers to the art-recognized assay described by Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997.

The term "MSP" (Methylation-specific PCR) refers to the art-recognized methylation assay described by Herman et al. *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996, and by US Patent No. 5,786,146.

The term "COBRA" (Combined Bisulfite Restriction Analysis) refers to the art-recognized methylation assay described by Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997.

The term "MCA" (Methylated CpG Island Amplification) refers to the methylation assay described by Toyota et al., *Cancer Res.* 59:2307-12, 1999, and in WO 00/26401A1.

The term "hybridization" is to be understood as a bond of an oligonucleotide to a complementary sequence along the lines of the Watson-Crick base pairings in the sample DNA, forming a duplex structure.

"Stringent hybridization conditions," as defined herein, involve hybridizing at 68°C in 5x SSC/5x Denhardt's solution/1.0% SDS, and washing in 0.2x SSC/0.1% SDS at room temperature, or involve the art-recognized equivalent thereof (e.g., conditions in which a hybridization is carried out at 60°C in 2.5 x SSC buffer, followed by several washing steps at 37°C in a low buffer concentration, and remains stable). Moderately stringent conditions, as defined herein, involve including washing in 3x SSC at 42°C, or the art-recognized equivalent thereof. The parameters of salt concentration and temperature can be varied to achieve the optimal level of identity between the probe and the target nucleic acid. Guidance regarding such conditions is available in the art, for example, by Sambrook et al., 1989, Molecular

Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; and Ausubel et al. (eds.), 1995, Current Protocols in Molecular Biology, (John Wiley & Sons, N.Y.) at Unit 2.10.

The terms "array SEQ ID NO," "composite array SEQ ID NO," or "composite array sequence" refer to a sequence, hypothetical or otherwise, consisting of a head-to-tail (5' to 3') linear composite of all individual contiguous sequences of a subject array (*e.g.*, a head-to-tail composite of SEQ ID NOS:1-71, in that order).

The terms "array SEQ ID NO node," "composite array SEQ ID NO node," or "composite array sequence node" refer to a *junction* between any two individual contiguous sequences of the "array SEQ ID NO," the "composite array SEQ ID NO," or the "composite array sequence."

In reference to composite array sequences, the phrase "contiguous nucleotides" refers to a contiguous sequence region of any individual contiguous sequence of the composite array, but does not include a region of the composite array sequence that includes a "node," as defined herein above.

Overview:

The present invention provides for molecular genetic markers that have novel utility for the analysis of methylation patterns associated with the development of colon cell proliferative disorders. Said markers may be used for detecting or distinguishing between colon cell proliferative disorders, thereby providing improved means for the classification and treatment of said disorders.

Bisulfite modification of DNA is an art-recognized tool used to assess CpG methylation status. 5-methylcytosine is the most frequent covalent base modification in the DNA of eukaryotic cells. It plays a role, for example, in the regulation of the transcription, in genetic imprinting, and in tumorigenesis. Therefore, the identification of 5-methylcytosine as a component of genetic information is of considerable interest. However, 5-methylcytosine positions cannot be identified by sequencing, because 5-methylcytosine has the same base pairing behavior as cytosine. Moreover, the epigenetic information carried by 5-methylcytosine is completely lost during, *e.g.*, PCR amplification.

The most frequently used method for analyzing DNA for the presence of 5-methylcytosine is based upon the specific reaction of bisulfite with cytosine whereby, upon subsequent alkaline hydrolysis, cytosine is converted to uracil which corresponds to thymine in its base pairing behavior. Significantly, however, 5-methylcytosine remains unmodified under these conditions. Consequently, the original DNA is *converted* in such a manner that methylcytosine, which originally could not be distinguished from cytosine by its hybridization behavior, can now be detected as the only remaining cytosine using standard, art-recognized molecular biological techniques, for example, by amplification and hybridization, or by sequencing. All of these techniques are based on differential base pairing properties, which can now be fully exploited.

The prior art, in terms of sensitivity, is defined by a method comprising enclosing the DNA to be analyzed in an agarose matrix, thereby preventing the diffusion and renaturation of the DNA (bisulfite only reacts with single-stranded DNA), and replacing all precipitation and purification steps with fast dialysis (Olek A, et al., A modified and improved method for bisulfite based cytosine methylation analysis, *Nucleic Acids Res.* 24:5064-6, 1996). It is thus possible to analyze individual cells for methylation status, illustrating the utility and sensitivity of the method. An overview of art-recognized methods for detecting 5-methylcytosine is provided by Rein, T., et al., *Nucleic Acids Res.*, 26:2255, 1998.

The bisulfite technique, barring few exceptions (*e.g.*, Zeschnigk M, et al., *Eur J Hum Genet.* 5:94-98, 1997), is currently only used in research. In all instances, short, specific fragments of a known gene are amplified subsequent to a bisulfite treatment, and either completely sequenced (Olek & Walter, *Nat Genet.* 1997 17:275-6, 1997), subjected to one or more primer extension reactions (Gonzalgo & Jones, *Nucleic Acids Res.*, 25:2529-31, 1997; WO 95/00669; U.S. Patent No. 6,251,594) to analyze individual cytosine positions, or treated by enzymatic digestion (Xiong & Laird, *Nucleic Acids Res.*, 25:2532-4, 1997). Detection by hybridization has also been described in the art (Olek et al., WO 99/28498). Additionally, use of the bisulfite technique for methylation detection with respect to individual genes has been described (Grigg & Clark, *Bioessays*, 16:431-6, 1994; Zeschnigk M, et al., *Hum Mol Genet.*,

6:387-95, 1997; Feil R, et al., *Nucleic Acids Res.*, 22:695-, 1994; Martin V, et al., *Gene*, 157:261-4, 1995; WO 9746705 and WO 9515373).

The present invention provides for the use of the bisulfite technique , in combination with one or more methylation assays, for determination of the methylation status of CpG dinuclotide sequences within sequences from the group consisting of SEQ ID NO:1 to SEQ ID NO:58. According to the present invention, determination of the methylation status of CpG dinuclotide sequences within sequences from the group consisting of SEQ ID NO:1 to SEQ ID NO:58 has diagnostic and prognostic utility.

Methylation Assay Procedures. Various methylation assay procedures are known in the art, and can be used in conjunction with the present invention. These assays allow for determination of the methylation state of one or a plurality of CpG dinucleotides (*e.g.*, CpG islands) within a DNA sequence. Such assays involve, among other techniques, DNA sequencing of bisulfite-treated DNA, PCR (for sequence-specific amplification), Southern blot analysis, and use of methylation-sensitive restriction enzymes.

For example, genomic sequencing has been simplified for analysis of DNA methylation patterns and 5-methylcytosine distribution by using bisulfite treatment (Frommer et al., *Proc. Natl. Acad. Sci. USA* 89:1827-1831, 1992). Additionally, restriction enzyme digestion of PCR products amplified from bisulfite-converted DNA is used, *e.g.*, the method described by Sadri & Hornsby (*Nucl. Acids Res.* 24:5058-5059, 1996), or COBRA (Combined Bisulfite Restriction Analysis) (Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997).

COBRA. COBRA analysis is a quantitative methylation assay useful for determining DNA methylation levels at specific gene loci in small amounts of genomic DNA (Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997). Briefly, restriction enzyme digestion is used to reveal methylation-dependent sequence differences in PCR products of sodium bisulfite-treated DNA. Methylation-dependent sequence differences are first introduced into the genomic DNA by standard bisulfite treatment according to the procedure described by Frommer et al. (*Proc. Natl. Acad. Sci. USA* 89:1827-1831, 1992). PCR amplification of the bisulfite converted DNA is then performed using primers specific for the interested CpG

islands, followed by restriction endonuclease digestion, gel electrophoresis, and detection using specific, labeled hybridization probes. Methylation levels in the original DNA sample are represented by the relative amounts of digested and undigested PCR product in a linearly quantitative fashion across a wide spectrum of DNA methylation levels. In addition, this technique can be reliably applied to DNA obtained from microdissected paraffin-embedded tissue samples. Typical reagents (*e.g.*, as might be found in a typical COBRA-based kit) for COBRA analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); restriction enzyme and appropriate buffer; gene-hybridization oligo; control hybridization oligo; kinase labeling kit for oligo probe; and radioactive nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery reagents or kits (*e.g.*, precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

Preferably, assays such as "MethyLight™" (a fluorescence-based real-time PCR technique) (Eads et al., *Cancer Res.* 59:2302-2306, 1999), Ms-SNuPE (Methylation-sensitive Single Nucleotide Primer Extension) reactions (Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997), methylation-specific PCR ("MSP"; Herman et al., *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996; US Patent No. 5,786,146), and methylated CpG island amplification ("MCA"; Toyota et al., *Cancer Res.* 59:2307-12, 1999) are used alone or in combination with other of these methods.

MethyLight ™. The MethyLight ™ assay is a high-throughput quantitative methylation assay that utilizes fluorescence-based real-time PCR (TaqMan ™) technology that requires no further manipulations after the PCR step (Eads et al., *Cancer Res.* 59:2302-2306, 1999). Briefly, the MethyLight ™ process begins with a mixed sample of genomic DNA that is converted, in a sodium bisulfite reaction, to a mixed pool of methylation-dependent sequence differences according to standard procedures (the bisulfite process converts unmethylated cytosine residues to uracil). Fluorescence-based PCR is then performed either in an "unbiased" (with primers that do not overlap known CpG methylation sites) PCR reaction, or in a "biased" (with PCR primers that overlap known CpG dinucleotides) reaction. Sequence discrimination can occur either at the level of the amplification process or at the level of the

fluorescence detection process, or both.

The MethyLight ™ assay may be used as a quantitative test for methylation patterns in the genomic DNA sample, wherein sequence discrimination occurs at the level of probe hybridization. In this quantitative version, the PCR reaction provides for unbiased amplification in the presence of a fluorescent probe that overlaps a particular putative methylation site. An unbiased control for the amount of input DNA is provided by a reaction in which neither the primers, nor the probe overlie any CpG dinucleotides. Alternatively, a qualitative test for genomic methylation is achieved by probing of the biased PCR pool with either control oligonucleotides that do not "cover" known methylation sites (a fluorescence-based version of the "MSP" technique), or with oligonucleotides covering potential methylation sites.

The MethyLight ™ process can by used with a "TaqMan®" probe in the amplification process. For example, double-stranded genomic DNA is treated with sodium bisulfite and subjected to one of two sets of PCR reactions using TaqMan® probes; *e.g.*, with either biased primers and TaqMan® probe, or unbiased primers and TaqMan® probe. The TaqMan® probe is dual-labeled with fluorescent "reporter" and "quencher" molecules, and is designed to be specific for a relatively high GC content region so that it melts out at about 10°C higher temperature in the PCR cycle than the forward or reverse primers. This allows the TaqMan® probe to remain fully hybridized during the PCR annealing/extension step. As the Taq polymerase enzymatically synthesizes a new strand during PCR, it will eventually reach the annealed TaqMan® probe. The Taq polymerase 5' to 3' endonuclease activity will then displace the TaqMan® probe by digesting it to release the fluorescent reporter molecule for quantitative detection of its now unquenched signal using a real-time fluorescent detection system.

Typical reagents (*e.g.*, as might be found in a typical MethyLight ™-based kit) for MethyLight ™ analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); TaqMan® probes; optimized PCR buffers and deoxynucleotides; and Taq polymerase.

Ms-SNuPE. The Ms-SNuPE technique is a quantitative method for assessing

methylation differences at specific CpG sites based on bisulfite treatment of DNA, followed by single-nucleotide primer extension (Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997). Briefly, genomic DNA is reacted with sodium bisulfite to convert unmethylated cytosine to uracil while leaving 5-methylcytosine unchanged. Amplification of the desired target sequence is then performed using PCR primers specific for bisulfite-converted DNA, and the resulting product is isolated and used as a template for methylation analysis at the CpG site(s) of interest. Small amounts of DNA can be analyzed (*e.g.*, microdissected pathology sections), and it avoids utilization of restriction enzymes for determining the methylation status at CpG sites.

Typical reagents (*e.g.*, as might be found in a typical Ms-SNuPE-based kit) for Ms-SNuPE analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); optimized PCR buffers and deoxynucleotides; gel extraction kit; positive control primers; Ms-SNuPE primers for specific gene; reaction buffer (for the Ms-SNuPE reaction); and radioactive nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery regents or kit (*e.g.*, precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

MSP. MSP (methylation-specific PCR) allows for assessing the methylation status of virtually any group of CpG sites within a CpG island, independent of the use of methylation-sensitive restriction enzymes (Herman et al. *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996; US Patent No. 5,786,146). Briefly, DNA is modified by sodium bisulfite converting all unmethylated, but not methylated cytosines to uracil, and subsequently amplified with primers specific for methylated versus unmethylated DNA. MSP requires only small quantities of DNA, is sensitive to 0.1% methylated alleles of a given CpG island locus, and can be performed on DNA extracted from paraffin-embedded samples. Typical reagents (*e.g.*, as might be found in a typical MSP-based kit) for MSP analysis may include, but are not limited to: methylated and unmethylated PCR primers for specific gene (or methylation-altered DNA sequence or CpG island), optimized PCR buffers and deoxynucleotides, and specific probes.

MCA. The MCA technique is a method that can be used to screen for altered

methylation patterns in genomic DNA, and to isolate specific sequences associated with these changes (Toyota et al., *Cancer Res.* 59:2307-12, 1999). Briefly, restriction enzymes with different sensitivities to cytosine methylation in their recognition sites are used to digest genomic DNAs from primary tumors, cell lines, and normal tissues prior to arbitrarily primed PCR amplification. Fragments that show differential methylation are cloned and sequenced after resolving the PCR products on high-resolution polyacrylamide gels. The cloned fragments are then used as probes for Southern analysis to confirm differential methylation of these regions. Typical reagents (*e.g.*, as might be found in a typical MCA-based kit) for MCA analysis may include, but are not limited to: PCR primers for arbitrary priming Genomic DNA; PCR buffers and nucleotides, restriction enzymes and appropriate buffers; gene-hybridization oligos or probes; control hybridization oligos or probes.

GENOMIC SEQUENCES ACCORDING TO SEQ ID NO:1 to SEQ ID NO:58, AND TREATED VARIANTS THEREOF ACCORDING TO SEQ ID NO:304 to SEQ ID NO:535, WERE DETERMINED TO HAVE UTILITY FOR THE DETECTION, CLASSIFICATION AND/OR TREATMENT OF COLON CELL PROLIFERATIVE DISORDERS

The present invention is based upon the analysis of methylation levels within one or more genomic sequences taken from the group consisting SEQ ID NO:1 to SEQ ID NO:58.

Particular embodiments of the present invention provide a novel application of the analysis of methylation levels and/or patterns within said sequences that enables a precise detection, characterisation and/or treatment of colon cell proliferative disorders. Early detection of colon cell proliferative disorders is directly linked with disease prognosis, and the disclosed method thereby enables the physician and patient to make better and more informed treatment decisions.

FURTHER IMPROVEMENTS

The present invention provides novel uses for genomic sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:58. Additional embodiments provide modified variants of SEQ ID NO:1 to SEQ ID NO:58, as well as oligonucleotides and/or

PNA-oligomers for analysis of cytosine methylation patterns within SEQ ID NO:1 to SEQ ID NO:58.

An objective of the invention comprises analysis of the methylation state of one or more CpG dinucleotides within at least one of the genomic sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO: 58 and sequences complementary thereto.

The disclosed invention provides treated nucleic acids, derived from genomic SEQ ID NO:1 to SEQ ID NO58, wherein the treatment is suitable to convert at least one unmethylated cytosine base of the genomic DNA sequence to uracil or another base that is detectably dissimilar to cytosine in terms of hybridization. The genomic sequences in question may comprise one, or more, consecutive or random methylated CpG positions. Said treatment preferrably comprises use of a reagent selected from the group consisting of bisulfite, hydrogen sulfite, disulfite, and combinations thereof. In a preferred embodiment of the invention, the objective comprises analysis of a modified nucleic acid comprising a sequence of at least 16 contiguous nucleotide bases in length of a sequence selected from the group consisting of SEQ ID NO:304 to SEQ ID NO:535, wherein said sequence comprises at least one CpG, TpA or CpA dinucleotide and sequences complementary thereto. The sequences of SEQ ID NO:304 to SEQ ID NO:535 provide modified versions of the nucleic acid according to SEQ ID NO:1 to SEQ ID NO:58, wherein the modification of each genomic sequence results in the synthesis of a nucleic acid having a sequence that is unique and distinct from said genomic sequence as follows. For each sense strand genomic DNA, *e.g.*, SEQ ID NO:1, four converted versions are disclosed. A first version wherein "C" →"T," but "CpG" remains "CpG" (*i.e.*, corresponds to case where, for the genomic sequence, all "C" residues of CpG dinucleotide sequences are methylated and are thus not converted); a second version discloses the complement of the disclosed genomic DNA sequence (i.e. *anti*sense strand), wherein "C" →"T," but "CpG" remains "CpG" (*i.e.*, corresponds to case where, for all "C" residues of CpG dinucleotide sequences are methylated and are thus not converted). The 'upmethylated' converted sequences of SEQ ID NO:1 to SEQ ID NO:58 correspond to SEQ ID NO:304 to SEQ ID NO:419. A third chemically converted version of each genomic sequences is provided, wherein "C" →"T" for all "C" residues, including those of "CpG" dinucleotide

sequences (*i.e.*, corresponds to case where, for the genomic sequences, all "C" residues of CpG dinucleotide sequences are unmethylated); a final chemically converted version of each sequence, discloses the complement of the disclosed genomic DNA sequence (i.e. *anti*sense strand), wherein "C" →"T" for all "C" residues, including those of "CpG" dinucleotide sequences (*i.e.*, corresponds to case where, for the complement (*anti*sense strand) of each genomic sequence, all "C" residues of CpG dinucleotide sequences are unmethylated). The 'downmethylated' converted sequences of SEQ ID NO:1 to SEQ ID NO:58 correspond to SEQ ID NO:420 to SEQ ID NO:535.

Significantly, heretofore, the nucleic acid sequences and molecules according to SEQ ID NOS:1 to SEQ ID NO:535 were not implicated in or connected with the detection, classification or treatment of colon cell proliferative disorders.

In an alternative preferred embodiment, such analysis comprises the use of an oligonucleotide or oligomer for detecting the cytosine methylation state within genomic or pretreated (chemically modified) DNA, according to SEQ ID NOS:1 to SEQ ID NO:535. Said oligonucleotide or oligomer comprising a nucleic acid sequence having a length of at least nine (9) nucleotides which hybridizes, under moderately stringent or stringent conditions (as defined herein above), to a pretreated nucleic acid sequence according to SEQ ID NOS:304 to SEQ ID NO:535 and/or sequences complementary thereto, or to a genomic sequence according to SEQ ID NOS:1 to SEQ ID NO:58 and/or sequences complementary thereto.

Thus, the present invention includes nucleic acid molecules (*e.g.*, oligonucleotides and peptide nucleic acid (PNA) molecules (PNA-oligomers)) that hybridize under moderately stringent and/or stringent hybridization conditions to all or a portion of the sequences SEQ ID NOS:1 to SEQ ID NO:535, or to the complements thereof. The hybridizing portion of the hybridizing nucleic acids is typically at least 9, 15, 20, 25, 30 or 35 nucleotides in length. However, longer molecules have inventive utility, and are thus within the scope of the present invention.

Preferably, the hybridizing portion of the inventive hybridizing nucleic acids is at least 95%, or at least 98%, or 100% identical to the sequence, or to a portion thereof of SEQ ID NOS:1 to SEQ ID NO:535, or to the complements thereof.

Hybridizing nucleic acids of the type described herein can be used, for example, as a primer (*e.g.*, a PCR primer), or a diagnostic and/or prognostic probe or primer. Preferably, hybridization of the oligonucleotide probe to a nucleic acid sample is performed under stringent conditions and the probe is 100% identical to the target sequence. Nucleic acid duplex or hybrid stability is expressed as the melting temperature or Tm, which is the temperature at which a probe dissociates from a target DNA. This melting temperature is used to define the required stringency conditions.

For target sequences that are related and substantially identical to the corresponding sequence of SEQ ID NOS:1 to SEQ ID NO:58 (such as allelic variants and SNPs), rather than identical, it is useful to first establish the lowest temperature at which only homologous hybridization occurs with a particular concentration of salt (*e.g.*, SSC or SSPE). Then, assuming that 1% mismatching results in a 1°C decrease in the Tm, the temperature of the final wash in the hybridization reaction is reduced accordingly (for example, if sequences having > 95% identity with the probe are sought, the final wash temperature is decreased by 5°C). In practice, the change in Tm can be between 0.5°C and 1.5°C per 1% mismatch.

Examples of inventive oligonucleotides of length X (in nucleotides), as indicated by polynucleotide positions with reference to, *e.g.*, SEQ ID NO:1, include those corresponding to sets (sense and antisense sets) of consecutively overlapping oligonucleotides of length X, where the oligonucleotides within each consecutively overlapping set (corresponding to a given X value) are defined as the finite set of Z oligonucleotides from nucleotide positions:

n to (n + (X-1));

where n=1, 2, 3,...(Y-(X-1));

where Y equals the length (nucleotides or base pairs) of SEQ ID NO:1 (2,280);

where X equals the common length (in nucleotides) of each oligonucleotide in the set (*e.g.*, X=20 for a set of consecutively overlapping 20-mers); and

where the number (Z) of consecutively overlapping oligomers of length X for a given SEQ ID NO of length Y is equal to Y-(X-1). For example Z= 2,280-19= 2,261 for either sense or antisense sets of SEQ ID NO:1, where X=20.

Preferably, the set is limited to those oligomers that comprise at least one CpG, TpG or CpA dinucleotide.

Examples of inventive 20-mer oligonucleotides include the following set of 2,261 oligomers (and the antisense set complementary thereto), indicated by polynucleotide positions with reference to SEQ ID NO:1:

1-20, 2-21, 3-22, 4-23, 5-24, ...2259-2278, 2260-2279 and 2261-2280.

Preferably, the set is limited to those oligomers that comprise at least one CpG, TpG or CpA dinucleotide.

Likewise, examples of inventive 25-mer oligonucleotides include the following set of 2,256 oligomers (and the antisense set complementary thereto), indicated by polynucleotide positions with reference to SEQ ID NO:1:

1-25, 2-26, 3-27, 4-28, 5-29, ...2254-2278, 2255-2279 and 2256-2280.

Preferably, the set is limited to those oligomers that comprise at least one CpG, TpG or CpA dinucleotide.

The present invention encompasses, for *each* of SEQ ID NOS:1 to SEQ ID NO:535 (sense and antisense), multiple consecutively overlapping sets of oligonucleotides or modified oligonucleotides of length X, where, *e.g.*, X= 9, 10, 17, 20, 22, 23, 25, 27, 30 or 35 nucleotides.

The oligonucleotides or oligomers according to the present invention constitute effective tools useful to ascertain genetic and epigenetic parameters of the genomic sequence corresponding to SEQ ID NOS:1 to SEQ ID NO:58. Preferred sets of such oligonucleotides or modified oligonucleotides of length X are those consecutively overlapping sets of oligomers corresponding to SEQ ID NOS:1 to SEQ ID NO:535 (and to the complements thereof). Preferably, said oligomers comprise at least one CpG, TpG or CpA dinucleotide.

Particularly preferred oligonucleotides or oligomers according to the present invention are those in which the cytosine of the CpG dinucleotide (or of the corresponding converted

TpG or CpA dinculeotide) sequences is within the middle third of the oligonucleotide; that is, where the oligonucleotide is, for example, 13 bases in length, the CpG, TpG or CpA dinucleotide is positioned within the fifth to ninth nucleotide from the 5'-end.

The oligonucleotides of the invention can also be modified by chemically linking the oligonucleotide to one or more moieties or conjugates to enhance the activity, stability or detection of the oligonucleotide. Such moieties or conjugates include chromophores, fluorophors, lipids such as cholesterol, cholic acid, thioether, aliphatic chains, phospholipids, polyamines, polyethylene glycol (PEG), palmityl moieties, and others as disclosed in, for example, United States Patent Numbers 5,514,758, 5,565,552, 5,567,810, 5,574,142, 5,585,481, 5,587,371, 5,597,696 and 5,958,773. The probes may also exist in the form of a PNA (peptide nucleic acid) which has particularly preferred pairing properties. Thus, the oligonucleotide may include other appended groups such as peptides, and may include hybridization-triggered cleavage agents (Krol et al., *BioTechniques* 6:958-976, 1988) or intercalating agents (Zon, *Pharm. Res.* 5:539-549, 1988). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a chromophore, fluorophor, peptide, hybridization-triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The oligonucleotide may also comprise at least one art-recognized modified sugar and/or base moiety, or may comprise a modified backbone or non-natural internucleoside linkage.

The oligonucleotides or oligomers according to particular embodiments of the present invention are typically used in 'sets,' which contain at least one oligomer for analysis of each of the CpG dinucleotides of genomic sequences SEQ ID NOS:1 to SEQ ID NO:58 and sequences complementary thereto, or to the corresponding CpG, TpG or CpA dinucleotide within a sequence of the pretreated nucleic acids according to SEQ ID NOS:304 to SEQ ID NO:535 and sequences complementary thereto. However, it is anticipated that for economic or other factors it may be preferable to analyze a limited selection of the CpG dinucleotides within said sequences, and the content of the set of oligonucleotides is altered accordingly.

Therefore, in particular embodiments, the present invention provides a set of at least two (2) (oligonucleotides and/or PNA-oligomers) useful for detecting the cytosine methylation state in pretreated genomic DNA (SEQ ID NOS:304 to SEQ ID NO:535), or in genomic DNA (SEQ ID NOS:1 to SEQ ID NO:58 and sequences complementary thereto). These probes enable diagnosis, classification and/or therapy of genetic and epigenetic parameters of colon cell proliferative disorders. The set of oligomers may also be used for detecting single nucleotide polymorphisms (SNPs) in pretreated genomic DNA (SEQ ID NOS:304 to SEQ ID NO:535), or in genomic DNA (SEQ ID NOS:1 to SEQ ID NO:58 and sequences complementary thereto).

In preferred embodiments, at least one, and more preferably all members of a set of oligonucleotides is bound to a solid phase.

In further embodiments, the present invention provides a set of at least two (2) oligonucleotides that are used as 'primer' oligonucleotides for amplifying DNA sequences of one of SEQ ID NOS:1 to SEQ ID NO:535 and sequences complementary thereto, or segments thereof.

It is anticipated that the oligonucleotides may constitute all or part of an "array" or "DNA chip" (*i.e.*, an arrangement of different oligonucleotides and/or PNA-oligomers bound to a solid phase). Such an array of different oligonucleotide- and/or PNA-oligomer sequences can be characterized, for example, in that it is arranged on the solid phase in the form of a rectangular or hexagonal lattice. The solid-phase surface may be composed of silicon, glass, polystyrene, aluminum, steel, iron, copper, nickel, silver, or gold. Nitrocellulose as well as plastics such as nylon, which can exist in the form of pellets or also as resin matrices, may also be used. An overview of the Prior Art in oligomer array manufacturing can be gathered from a special edition of Nature Genetics (*Nature Genetics Supplement*, Volume 21, January 1999, and from the literature cited therein). Fluorescently labeled probes are often used for the scanning of immobilized DNA arrays. The simple attachment of Cy3 and Cy5 dyes to the 5'-OH of the specific probe are particularly suitable for fluorescence labels. The detection of the fluorescence of the hybridized probes may be carried out, for example, via a confocal microscope. Cy3 and Cy5 dyes, besides many others, are commercially available.

It is also anticipated that the oligonucleotides, or particular sequences thereof, may constitute all or part of an "virtual array" wherein the oligonucleotides, or particular sequences thereof, are used, for example, as 'specifiers' as part of, or in combination with a diverse population of unique labeled probes to analyze a complex mixture of analytes. Such a method, for example is described in US 2003/0013091 (United States serial number 09/898,743, published 16 January 2003). In such methods, enough labels are generated so that each nucleic acid in the complex mixture (*i.e.*, each analyte) can be uniquely bound by a unique label and thus detected (each label is directly counted, resulting in a digital read-out of each molecular species in the mixture).

It is particularly preferred that the oligomers according to the invention are utilised for at least one of: detection of; detection and differentiation between or among subclasses of; diagnosis of; prognosis of; treatment of; monitoring of; and treatment and monitoring of colon cell proliferative disorders. This is enabled by use of said sets for the detection or detection and differentiation of one or more of the following classes of tissues: colorectal carcinoma, colon adenoma, inflammatory colon tissue, grade 2 dysplasia colon adenomas less than 1 cm, grade 3 dysplasia colon adenomas larger than 1 cm, normal colon tissue, non-colon healthy tissue and non-colon cancer tissue.

Particularly preferrred are those sets of oligomer that comprise at least two oligonucleotides selected from one of the following sets of oligonucleotides:

SEQ ID NOS:59 - 285;

SEQ ID 59 - 109, 113 - 223, 227 - 293;

SEQ ID 59 - 109, 113 - 161, 164 - 223, 227 - 285, 287 - 293;

SEQ ID 89, 90, 126 - 135, 147 - 151, 224 - 226, 253 - 256, 261 - 267, 283 - 285;

SEQ ID 59 - 161, 164 - 293;

SEQ ID 59 - 109, 113 - 299;

SEQ ID 59 - 109, 113 - 293, 296 – 299;

SEQ ID NOS:1-12, 15-20, 22, 25-36, 38-49, 51-58;

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003, 1010 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142;

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 885, 890 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003, 1010 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074,

1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1124, 1129 - 1141, 1141, 1142, 1142;

SEQ ID NOS:738 - 740, 810 - 814, 814, 815, 815 - 829, 854 - 865, 1004 - 1006, 1006, 1007, 1007 - 1009, 1062, 1062, 1063, 1063 - 1069, 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1091, 1121 - 1124;

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142 - 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152 - 1154, 1154, 1155, 1155, 1156, 1156, 1157, 1157, 1158, 1158, 1159, 1159;

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 885,

890 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142;

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142 - 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152;

and SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142, 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152.

In one embodiment of the method, at least one of colorectal carcinoma tissue or colon adenomas is distinguished from at least one tissue selected from the group consisting of inflammatory colon tissue and normal colon tissue, by use of a set comprising of at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 285; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957,

957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1124.

In one embodiment of the method, colorectal carcinoma is distinguished from at least one tissue selected from the group consisting of non-colon healthy tissue, peripheral blood lymphocytes and non-colon cancer of by use of a set comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 285; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087

- 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 – 1124.

In one embodiment of the method, the differentiation of is enabled by use of a set comprising of at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 109, 113 - 223, 227 - 293; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003, 1010 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142.

In one embodiment of the method, colorectal carcinoma is distinguished from at least one tissue selected from the group consisting of inflammatory colon tissue, normal colon tissue, non-colon healthy tissue, peripheral blood lymphocytes, colon adenomas and non-colon cancer tissue. by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 109, 113 - 161, 164 - 223, 227 - 285, 287 - 293; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726,

726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 885, 890 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003, 1010 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1124, 1129 - 1141, 1141, 1142, 1142.

In one embodiment of the method, the colorectal carcinoma is distinguished from colon adenomas by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:89, 90, 126 - 135, 147 - 151, 224 - 226, 253 - 256, 261 - 267, 283 - 285; and

SEQ ID NOS:738 - 740, 810 - 814, 814, 815, 815 - 829, 854 - 865, 1004 - 1006, 1006, 1007, 1007 - 1009, 1062, 1062, 1063, 1063 - 1069, 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1091, 1121 - 1124.

In one embodiment of the method, at least one of colorectal carcinoma tissue, or colon adenomas is distinguished from at least one tissue selected from the group consisting of inflammatory colon tissue and normal colon tissue is enabled by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 303; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726,

726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142 - 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152 - 1154, 1154, 1155, 1155, 1156, 1156, 1157, 1157, 1158, 1158, 1159, 1159.

In one embodiment of the method, colorectal carcinoma tissue is distinguished from at least one of inflammatory colon tissue and normal colon tissue by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 161, 164 - 293; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 885, 890 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994,

994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142.

In one embodiment of the method, at least one of colorectal carcinoma tissue, or colon adenomas is distinguished from at least one tissue selected from the group consisting of inflammatory colon tissue, normal colon tissue, non-colon healthy tissue, peripheral blood lymphocytes, and non-colon cancer tissue by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ IDNOS:59 - 109, 113 - 299; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114,

1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142 - 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152.

In one embodiment of the method, tissues originating from the colon are distinguished from tissues of non-colon origin by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 303; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142 - 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152 - 1154, 1154, 1155, 1155, 1156, 1156, 1157, 1157, 1158, 1158, 1159, 1159.

In one embodiment of the method, cell proliferative disorders are distinguished from healthy tissues by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 109, 113 - 293, 296 - 299; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142, 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152.

The present invention further provides a method for ascertaining genetic and/or epigenetic parameters of the genomic sequences according to SEQ ID NOS:1 to SEQ ID NO:58 within a subject by analyzing cytosine methylation and single nucleotide polymorphisms. Said method comprising contacting a nucleic acid comprising one or more of SEQ ID NOS:1 to SEQ ID NO:58 in a biological sample obtained from said subject with at least one reagent or a series of reagents, wherein said reagent or series of reagents, distinguishes between methylated and non-methylated CpG dinucleotides within the target nucleic acid.

Preferably, said method comprises the following steps: In the *first step*, a sample of the tissue to be analysed is obtained. The source may be any suitable source, such as cell lines, histological slides, biopsies, tissue embedded in paraffin, bodily fluids, ejaculate, urine, blood and all possible combinations thereof. The DNA is then isolated from the sample. Extraction

may be by means that are standard to one skilled in the art, including the use of commercially available kits, detergent lysates, sonification and vortexing with glass beads. Once the nucleic acids have been extracted, the genomic double stranded DNA is used in the analysis.

In the *second step* of the method, the genomic DNA sample is treated in such a manner that cytosine bases which are unmethylated at the 5'-position are converted to uracil, thymine, or another base which is dissimilar to cytosine in terms of hybridization behavior. This will be understood as 'pretreatment' or 'treatment' herein.

The above-described treatment of genomic DNA is preferably carried out with bisulfite (hydrogen sulfite, disulfite) and subsequent alkaline hydrolysis which results in a conversion of non-methylated cytosine nucleobases to uracil or to another base which is dissimilar to cytosine in terms of base pairing behavior.

In the *third step* of the method, fragments of the pretreated DNA are amplified, using sets of primer oligonucleotides according to the present invention, and an amplification enzyme. The amplification of several DNA segments can be carried out simultaneously in one and the same reaction vessel. Typically, the amplification is carried out using a polymerase chain reaction (PCR). The set of primer oligonucleotides includes at least two oligonucleotides whose sequences are each reverse complementary, identical, or hybridize under stringent or highly stringent conditions to an at least 16-base-pair long segment of the base sequences of one or more of SEQ ID NOS:304 to SEQ ID NO:535 and sequences complementary thereto.

In an alternate embodiment of the method, the methylation status of preselected CpG positions within the nucleic acid sequences comprising one or more of SEQ ID NOS:1 to SEQ ID NO:58 may be detected by use of methylation-specific primer oligonucleotides. This technique (MSP) has been described in United States Patent No. 6,265,171 to Herman. The use of methylation status specific primers for the amplification of bisulfite treated DNA allows the differentiation between methylated and unmethylated nucleic acids. MSP primers pairs contain at least one primer which hybridizes to a bisulfite treated CpG dinucleotide. Therefore, the sequence of said primers comprises at least one CpG dinucleotide. MSP primers specific for non-methylated DNA contain a "T' at the 3' position of the C position in

the CpG. Preferably, therefore, the base sequence of said primers is required to comprise a sequence having a length of at least 9 nucleotides which hybridizes to a pretreated nucleic acid sequence according to one of SEQ ID NOS:304 to SEQ ID NO:535 and sequences complementary thereto, wherein the base sequence of said oligomers comprises at least one CpG dinucleotide.

In a further preferred embodiment of the method, the MSP primers are selected from the group consisting SEQ ID NOS:1160, 1163, 1166, 1169, 1171, 1172, 1173, 1174, 1175, 1178, 1179, 1183, 1184, 1161, 1164, 1167, 1168, 1176, 1180, 1182, 1185, 1186, 1190, 1191, 1192, 1195, 1196, 1199, 1200, 1203, 1205, 1206, 1208, 1209, 1211, 1213, 1214, 1216, 1219, 1221, 1223, 1225, 1230, 1234, 1240, 1241, 1242, 1245, 1247, 1249, 1252, 1257, 1258, 1260, 1264, 1265, 1266, 1267, 1271, 1273, 1274, 1275, 1277, 1280, 1281, 1282, 1287, 1288, 1289, 1293, 1294, 1295, 1296, 1299, 1301, 1304, 1306, 1308, 1310, 1312, 1320, 1321, 1323, 1324, 1327, 1329, 1331, 1333, 1336, 1339, 1340, 1341, 1348, 1350, 1353, 1357, 1359, 1361, 1366, 1367, 1371, 1374, 1375, 1376, 1379, 1381, 1384, 1385, 1386, 1389, 1390, 1393, 1394, 1398, 1402, 1405, 1408, 1413, 1416, 1419, 1420, 1422, 1423, 1429, 1431, 1435, 1436, 1437, 1440, 1442, 1444, 1446, 1447, 1449, 1451, 1454, 1456, 1459, 1460, 1461, 1464, 1466, 1468, 1471, 1473, 1474, 1479, 1480, 1481, 1482, 1483, 1488, 1490, 1493, 1494, 1495, 1505, 1506, 1508, 1510, 1513, 1515, 1519, 1522, 1523, 1524, 1526, 1527, 1528, 1531, 1532, 1533, 1535, 1536, 1539, 1540, 1542, 1544, 1548, 1551, 1553, 1554, 1555, 1558, 1559, 1564, 1567, 1569, 1572, 1573, 1576, 1187, 1189, 1193, 1194, 1197, 1198, 1201, 1204, 1207, 1210, 1212, 1215, 1217, 1220, 1222, 1224, 1226, 1227, 1228, 1229, 1231, 1232, 1233, 1235, 1237, 1239, 1243, 1246, 1248, 1250, 1251, 1253, 1254, 1255, 1256, 1259, 1261, 1263, 1268, 1270, 1272, 1276, 1278, 1283, 1285, 1286, 1290, 1291, 1292, 1297, 1298, 1300, 1302, 1305, 1309, 1311, 1313, 1314, 1315, 1316, 1317, 1318, 1319, 1322, 1325, 1330, 1332, 1334, 1335, 1337, 1342, 1344, 1346, 1347, 1349, 1351, 1352, 1354, 1356, 1358, 1360, 1362, 1364, 1365, 1368, 1370, 1372, 1377, 1378, 1380, 1382, 1387, 1388, 1391, 1395, 1396, 1397, 1399, 1400, 1401, 1403, 1404, 1406, 1409, 1410, 1412, 1414, 1415, 1417, 1421, 1424, 1426, 1427, 1428, 1430, 1432, 1433, 1438, 1441, 1443, 1445, 1448, 1450, 1455, 1457, 1462, 1463, 1465, 1467, 1469, 1472, 1475, 1477, 1484, 1485, 1486, 1487, 1489, 1491, 1496, 1497, 1498, 1499, 1500, 1501, 1502, 1504, 1507,

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A further preferred embodiment of the method comprises the use of *blocker* oligonucleotides. The use of such blocker oligonucleotides has been described by Yu et al., *BioTechniques* 23:714-720, 1997. Blocking probe oligonucleotides are hybridized to the bisulfite treated nucleic acid concurrently with the PCR primers. PCR amplification of the nucleic acid is terminated at the 5' position of the blocking probe, such that amplification of a nucleic acid is suppressed where the complementary sequence to the blocking probe is present. The probes may be designed to hybridize to the bisulfite treated nucleic acid in a methylation status specific manner. For example, for detection of methylated nucleic acids within a population of unmethylated nucleic acids, suppression of the amplification of nucleic acids which are unmethylated at the position in question would be carried out by the use of blocking probes comprising a 'CpA' or 'TpA' at the position in question, as opposed to a 'CpG' if the suppression of amplification of methylated nucleic acids is desired.

For PCR methods using blocker oligonucleotides, efficient disruption of polymerase-mediated amplification requires that blocker oligonucleotides not be elongated by the

polymerase. Preferably, this is achieved through the use of blockers that are 3'-deoxyoligonucleotides, or oligonucleotides derivitized at the 3' position with other than a "free" hydroxyl group. For example, 3'-O-acetyl oligonucleotides are representative of a preferred class of blocker molecule.

Additionally, polymerase-mediated decomposition of the blocker oligonucleotides should be precluded. Preferably, such preclusion comprises either use of a polymerase lacking 5'-3' exonuclease activity, or use of modified blocker oligonucleotides having, for example, thioate bridges at the 5'-terminii thereof that render the blocker molecule nuclease-resistant. Particular applications may not require such 5' modifications of the blocker. For example, if the blocker- and primer-binding sites overlap, thereby precluding binding of the primer (*e.g.*, with excess blocker), degradation of the blocker oligonucleotide will be substantially precluded. This is because the polymerase will not extend the primer toward, and through (in the 5'-3' direction) the blocker—a process that normally results in degradation of the hybridized blocker oligonucleotide.

A particularly preferred blocker/PCR embodiment, for purposes of the present invention and as implemented herein, comprises the use of peptide nucleic acid (PNA) oligomers as blocking oligonucleotides. Such PNA blocker oligomers are ideally suited, because they are neither decomposed nor extended by the polymerase.

Preferably, therefore, the base sequence of said *blocking oligonucleotides* is required to comprise a sequence having a length of at least 9 nucleotides which hybridizes to a pretreated nucleic acid sequence according to one of SEQ ID NOS:304 to SEQ ID NO:535 and sequences complementary thereto, wherein the base sequence of said oligonucleotides comprises at least one CpG, TpG or CpA dinucleotide.

The fragments obtained by means of the amplification can carry a directly or indirectly detectable label. Preferred are labels in the form of fluorescence labels, radionuclides, or detachable molecule fragments having a typical mass which can be detected in a mass spectrometer. Where said labels are mass labels, it is preferred that the labeled amplificates have a single positive or negative net charge, allowing for better detectability in the mass spectrometer. The detection may be carried out and visualized by means of, *e.g.*, matrix

assisted laser desorption/ionization mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).

Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-TOF) is a very efficient development for the analysis of biomolecules (Karas & Hillenkamp, *Anal Chem.*, 60:2299-301, 1988). An analyte is embedded in a light-absorbing matrix. The matrix is evaporated by a short laser pulse thus transporting the analyte molecule into the vapour phase in an unfragmented manner. The analyte is ionized by collisions with matrix molecules. An applied voltage accelerates the ions into a field-free flight tube. Due to their different masses, the ions are accelerated at different rates. Smaller ions reach the detector sooner than bigger ones. MALDI-TOF spectrometry is well suited to the analysis of peptides and proteins. The analysis of nucleic acids is somewhat more difficult (Gut & Beck, *Current Innovations and Future Trends*, 1:147-57, 1995). The sensitivity with respect to nucleic acid analysis is approximately 100-times less than for peptides, and decreases disproportionally with increasing fragment size. Moreover, for nucleic acids having a multiply negatively charged backbone, the ionization process via the matrix is considerably less efficient. In MALDI-TOF spectrometry, the selection of the matrix plays an eminently important role. For desorption of peptides, several very efficient matrixes have been found which produce a very fine crystallisation. There are now several responsive matrixes for DNA, however, the difference in sensitivity between peptides and nucleic acids has not been reduced. This difference in sensitivity can be reduced, however, by chemically modifying the DNA in such a manner that it becomes more similar to a peptide. For example, phosphorothioate nucleic acids, in which the usual phosphates of the backbone are substituted with thiophosphates, can be converted into a charge-neutral DNA using simple alkylation chemistry (Gut & Beck, *Nucleic Acids Res.* 23: 1367-73, 1995). The coupling of a charge tag to this modified DNA results in an increase in MALDI-TOF sensitivity to the same level as that found for peptides. A further advantage of charge tagging is the increased stability of the analysis against impurities, which makes the detection of unmodified substrates considerably more difficult.

In the *fourth step* of the method, the amplificates obtained during the third step of the method are analysed in order to ascertain the methylation status of the CpG dinucleotides prior to the treatment.

In embodiments where the amplificates were obtained by means of MSP amplification, the presence or absence of an amplificate is in itself indicative of the methylation state of the CpG positions covered by the primer, according to the base sequences of said primer.

Amplificates obtained by means of both standard and methylation specific PCR may be further analyzed by means of hybridization-based methods such as, but not limited to, array technology and probe based technologies as well as by means of techniques such as sequencing and template directed extension.

In one embodiment of the method, the amplificates synthesised in *step three* are subsequently hybridized to an array or a set of oligonucleotides and/or PNA probes. In this context, the hybridization takes place in the following manner: the set of probes used during the hybridization is preferably composed of at least 2 oligonucleotides or PNA-oligomers; in the process, the amplificates serve as probes which hybridize to oligonucleotides previously bonded to a solid phase; the non-hybridized fragments are subsequently removed; said oligonucleotides contain at least one base sequence having a length of at least 9 nucleotides which is reverse complementary or identical to a segment of the base sequences specified in the present Sequence Listing; and the segment comprises at least one CpG , TpG or CpA dinucleotide.

In a preferred embodiment, said dinucleotide is present in the central third of the oligomer. For example, wherein the oligomer comprises one CpG dinucleotide, said dinucleotide is preferably the fifth to ninth nucleotide from the 5'-end of a 13-mer. One oligonucleotide exists for the analysis of each CpG dinucleotide within the sequence according to SEQ ID NOs:1 to SEQ ID NO 58, and the equivalent positions within SEQ ID NOS:304 to SEQ ID NO 535. Said oligonucleotides may also be present in the form of peptide nucleic acids. The non-hybridized amplificates are then removed.The hybridized amplificates are then detected. In this context, it is preferred that labels attached to the

amplificates are identifiable at each position of the solid phase at which an oligonucleotide sequence is located.

In yet a further embodiment of the method, the genomic methylation status of the CpG positions may be ascertained by means of oligonucleotide probes that are hybridised to the bisulfite treated DNA concurrently with the PCR amplification primers (wherein said primers may either be methylation specific or standard).

A particularly preferred embodiment of this method is the use of fluorescence-based Real Time Quantitative PCR (Heid et al., *Genome Res.* 6:986-994, 1996; *also see* United States Patent No. 6,331,393) employing a dual-labeled fluorescent oligonucleotide probe (TaqMan™ PCR, using an ABI Prism 7700 Sequence Detection System, Perkin Elmer Applied Biosystems, Foster City, California). The TaqMan™ PCR reaction employs the use of a nonextendible interrogating oligonucleotide, called a TaqMan™ probe, which, in preferred imbodiments, is designed to hybridize to a GpC-rich sequence located between the forward and reverse amplification primers. The TaqMan™ probe further comprises a fluorescent "reporter moiety" and a "quencher moiety" covalently bound to linker moieties (*e.g.*, phosphoramidites) attached to the nucleotides of the TaqMan™ oligonucleotide. For analysis of methylation within nucleic acids subsequent to bisulfite treatment, it is required that the probe be methylation specific, as described in United States Patent No. 6,331,393, (hereby incorporated by reference in its entirety) also known as the MethylLight™ assay. Variations on the TaqMan™ detection methodology that are also suitable for use with the described invention include the use of dual-probe technology (Lightcycler™) or fluorescent amplification primers (Sunrise™ technology). Both these techniques may be adapted in a manner suitable for use with bisulfite treated DNA, and moreover for methylation analysis within CpG dinucleotides.

A further suitable method for the use of probe oligonucleotides for the assessment of methylation by analysis of bisulfite treated nucleic acids In a further preferred embodiment of the method, the *fifth step* of the method comprises the use of template-directed oligonucleotide extension, such as MS-SNuPE as described by Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997.

In yet a further embodiment of the method, the *fifth step* of the method comprises sequencing and subsequent sequence analysis of the amplificate generated in the *third step* of the method (Sanger F., et al., *Proc Natl Acad Sci USA* 74:5463-5467, 1977).

Best mode

In the most preferred embodiment of the method the nucleic acids according to SEQ ID NO: 1 to SEQ ID NO 58 are isolated and treated according to the first three steps of the method outlined above, namely:

a) obtaining, from a subject, a biological sample having subject genomic DNA;

b) extracting or otherwise isolating the genomic DNA;

c) treating the genomic DNA of b), or a fragment thereof, with one or more reagents to convert cytosine bases that are unmethylated in the 5-position thereof to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties; and wherein

d) amplifying subsequent to treatment in c) is carried out in a methylation specific manner, namely by use of methylation specific primers or *blocking oligonucleotides*, and further wherein

e) detecting of the amplificates is carried out by means of a real-time detection probes, as described above.

Wherein the subsequent amplification of c) is carried out by means of methylation specific primers, as described above, said methylation specific primers comprise a sequence having a length of at least 9 nucleotides which hybridizes to a pretreated nucleic acid sequence according to one of SEQ ID NOs:304 to SEQ ID NO:535 and sequences complementary thereto, wherein the base sequence of said oligomers comprises at least one CpG dinucleotide. In a further preferred embodiment of the method the MSP primers are selected from the group consisting SEQ ID NOS:1160, 1163, 1166, 1169, 1171, 1172, 1173, 1174, 1175, 1178, 1179, 1183, 1184, 1161, 1164, 1167, 1168, 1176, 1180, 1182, 1185, 1186, 1190, 1191, 1192, 1195, 1196, 1199, 1200, 1203, 1205, 1206, 1208, 1209, 1211, 1213, 1214, 1216, 1219, 1221, 1223, 1225, 1230, 1234, 1240, 1241, 1242, 1245, 1247, 1249, 1252, 1257,

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8358, 8360, 8362, 8363, 8365, 8368, 8370, 8375, 8378, 8379, 8381, 8383, 8386, 8387, 8388, 8391, 8394, 8396, 8397, 8400, 8401, 8402, 8403, 8405, 8406, 8410, 8412, 8414, 8415, 8421, 8424, 8429, 8432, 8434, 8435, 8179, 8184, 8191, 8200, 8215, 8226, 8228, 8231, 8235, 8244, 8247, 8251, 8254, 8258, 8266, 8272, 8277, 8281, 8289, 8312, 8327, 8348, 8357, 8361, 8366, 8369, 8371, 8376, 8392, 8395, 8416, 8425.

Step e) of the method, namely the detection of the specific amplificates indicative of the methylation status of one or more CpG positions according to SEQ ID NOS:1 to SEQ ID NO:8 is carried out by means of real-time detection methods as described above, and wherein the sequence of said hybridization probes is selected from the group consisting SEQ ID NOS:1162, 1165, 1170, 1177, 1181, 1188, 1202, 1218, 1236, 1238, 1244, 1262, 1269, 1279, 1284, 1303, 1307, 1326, 1328, 1338, 1343, 1345, 1355, 1363, 1369, 1373, 1383, 1392, 1407, 1411, 1418, 1425, 1434, 1439, 1452, 1453, 1458, 1470, 1476, 1478, 1492, 1503, 1517, 1520, 1530, 1534, 1545, 1550, 1552, 1556, 1560, 1565, 1579, 1582, 1585, 1590, 1598, 1614, 1615, 1620, 1637, 1640, 1642, 1651, 1656, 1659, 1662, 1670, 1672, 1680, 1682, 1688, 1697, 1708, 1711, 1714, 1718, 1722, 1731, 1739, 1742, 1754, 1763, 1774, 1778, 1782, 1785, 1800, 1805, 1809, 1822, 1826, 1835, 1847, 1850, 1860, 1869, 1876, 1880, 1889, 1894, 1897, 1904, 1910, 1921, 1924, 1943, 1981, 1984, 1991, 2000, 2003, 2017, 2026, 2030, 2035, 2040, 2044, 2051, 2060, 2072, 2076, 2101, 2103, 2106, 2109, 2117, 2120, 2145, 2159, 2163, 2175, 2188, 2204, 2213, 2222, 2239, 2253, 2256, 2268, 2279, 2285, 2288, 2293, 2298, 2302, 2305, 2311, 2315, 2337, 2346, 2352, 2356, 2359, 2366, 2374, 2381, 2384, 2388, 2406, 2410, 2427, 2430, 2451, 2465, 2471, 2477, 2524, 2529, 2539, 2552, 2563, 2566, 2571, 2576, 2578, 2585, 2598, 2606, 2614, 2616, 2621, 2635, 2646, 2650, 2653, 2671, 2675, 2678, 2679, 2682, 2687, 2691, 2703, 2706, 2718, 2723, 2732, 2740, 2754, 2756, 2761, 2764, 2768, 2778, 2787, 2794, 2809, 2831, 2837, 2844, 2849, 2852, 2857, 2862, 2868, 2870, 2874, 2878, 2882, 2891, 2898, 2903, 2906, 2912, 2919, 2941, 2961, 2964, 2970, 2976, 2979, 2990, 2994, 3008, 3014, 3021, 3027, 3037, 3040, 3042, 3045, 3050, 3054, 3058, 3062, 3083, 3091, 3097, 3103, 3106, 3122, 3134, 3143, 3187, 3193, 3195, 3197, 3200, 3204, 3213, 3225, 3244, 3247, 3270, 3273, 3276, 3280, 3285, 3290, 3301, 3313, 3317, 3322, 3325, 3329, 3332, 3334, 3337, 3342, 3350, 3354, 3357, 3361, 3365, 3368, 3376, 3381, 3385, 3388, 3398, 3411, 3414, 3430, 3439, 3442, 3446, 3453, 3461,

3464, 3473, 3484, 3494, 3504, 3507, 3511, 3516, 3529, 3537, 3541, 3548, 3551, 3555, 3569, 3577, 3580, 3587, 3592, 3597, 3614, 3618, 3622, 3627, 3631, 3633, 3636, 3638, 3642, 3648, 3651, 3656, 3675, 3677, 3683, 3686, 3691, 3711, 3723, 3727, 3732, 3756, 3763, 3770, 3774, 3791, 3796, 3803, 3806, 3834, 3844, 3852, 3856, 3883, 3888, 3896, 3899, 3904, 3906, 3909, 3911, 3923, 3936, 3940, 3944, 3958, 3975, 3987, 3990, 3994, 3997, 4000, 4006, 4012, 4024, 4028, 4034, 4039, 4042, 4051, 4055, 4058, 4060, 4079, 4089, 4095, 4101, 4105, 4116, 4124, 4138, 4141, 4145, 4153, 4156, 4162, 4173, 4176, 4181, 4185, 4191, 4198, 4201, 4208, 4210, 4213, 4220, 4225, 4228, 4233, 4238, 4248, 4251, 4262, 4265, 4268, 4284, 4290, 4293, 4303, 4309, 4321, 4323, 4324, 4334, 4336, 4340, 4345, 4351, 4354, 4358, 4363, 4368, 4373, 4376, 4386, 4392, 4407, 4410, 4414, 4420, 4437, 4442, 4474, 4477, 4498, 4524, 4526, 4541, 4543, 4549, 4565, 4568, 4571, 4600, 4607, 4614, 4618, 4629, 4635, 4641, 4652, 4665, 4669, 4674, 4677, 4685, 4688, 4691, 4695, 4698, 4701, 4704, 4708, 4714, 4719, 4724, 4728, 4733, 4736, 4739, 4746, 4751, 4757, 4759, 4783, 4797, 4802, 4811, 4818, 4833, 4841, 4848, 4863, 4872, 4880, 4882, 4888, 4899, 4903, 4907, 4910, 4925, 4930, 4933, 4940, 4950, 4955, 4962, 4979, 4986, 4989, 4991, 4995, 5002, 5007, 5011, 5016, 5028, 5035, 5044, 5058, 5068, 5078, 5081, 5084, 5088, 5094, 5119, 5125, 5128, 5135, 5152, 5189, 5195, 5212, 5215, 5218, 5222, 5226, 5236, 5241, 5246, 5258, 5260, 5263, 5271, 5274, 5277, 5280, 5283, 5285, 5289, 5293, 5300, 5312, 5325, 5328, 5334, 5344, 5348, 5358, 5380, 5398, 5430, 5437, 5440, 5443, 5446, 5454, 5470, 5480, 5496, 5503, 5510, 5513, 5517, 5523, 5550, 5557, 5564, 5573, 5576, 5581, 5586, 5590, 5598, 5600, 5609, 5611, 5616, 5621, 5624, 5627, 5632, 5634, 5637, 5639, 5643, 5653, 5655, 5660, 5664, 5672, 5679, 5690, 5697, 5711, 5717, 5735, 5741, 5749, 5760, 5781, 5795, 5799, 5811, 5822, 5864, 5871, 5875, 5878, 5884, 5895, 5898, 5902, 5912, 5917, 5923, 5928, 5940, 5962, 5971, 5986, 5988, 6009, 6015, 6020, 6027, 6041, 6049, 6052, 6062, 6066, 6087, 6089, 6100, 6105, 6112, 6124, 6147, 6153, 6157, 6167, 6168, 6169, 6180, 6186, 6199, 6205, 6211, 6217, 6257, 6262, 6266, 6270, 6276, 6283, 6286, 6296, 6299, 6301, 6304, 6309, 6313, 6321, 6326, 6341, 6346, 6353, 6356, 6359, 6379, 6382, 6394, 6397, 6434, 6438, 6441, 6444, 6450, 6453, 6456, 6460, 6464, 6471, 6475, 6477, 6507, 6526, 6536, 6556, 6561, 6574, 6577, 6602, 6608, 6610, 6619, 6621, 6626, 6658, 6666, 6688, 6692, 6696, 6710, 6744, 6746, 6758, 6763, 6771, 6781, 6785, 6796, 6801, 6804, 6810, 6835, 6838, 6854, 6857, 6864, 6870, 6872,

6876, 6882, 6887, 6893, 6896, 6916, 6926, 6929, 6936, 6949, 6954, 6956, 6958, 6972, 6977, 6988, 6992, 7017, 7020, 7030, 7087, 7094, 7102, 7106, 7116, 7119, 7126, 7130, 7133, 7137, 7144, 7154, 7162, 7174, 7192, 7209, 7212, 7222, 7234, 7240, 7243, 7247, 7250, 7254, 7258, 7262, 7264, 7272, 7276, 7282, 7285, 7294, 7296, 7298, 7308, 7314, 7326, 7331, 7339, 7351, 7363, 7365, 7381, 7394, 7396, 7399, 7401, 7406, 7410, 7416, 7418, 7430, 7436, 7441, 7447, 7450, 7462, 7468, 7479, 7483, 7513, 7521, 7578, 7581, 7588, 7597, 7600, 7603, 7622, 7631, 7638, 7649, 7652, 7668, 7674, 7679, 7682, 7691, 7696, 7706, 7715, 7717, 7719, 7732, 7743, 7767, 7772, 7776, 7779, 7783, 7797, 7803, 7813, 7820, 7823, 7831, 7834, 7844, 7847, 7854, 7866, 7874, 7884, 7892, 7895, 7906, 7913, 7931, 7942, 7947,7951, 7954, 7957, 7960, 7965, 7968, 7975, 7978, 7989, 7993, 8013, 8036, 8045, 8048, 8053, 8062, 8069, 8073, 8077, 8079, 8081, 8084, 8087, 8092, 8094, 8097, 8101, 8110, 8113, 8116, 8121, 8145, 8147, 8152, 8161, 8164, 8179, 8184, 8191, 8200, 8215, 8226, 8228, 8231, 8235, 8244, 8247, 8251, 8254, 8258, 8266, 8272, 8277, 8281, 8289, 8312, 8327, 8348, 8357, 8361, 8366, 8369, 8371, 8376, 8392, 8395, 8416, and SEQ ID NO:8425.

Suitable combinations of methylation specific primers and methylation real-time detection probes are shown in TABLE 3, herein below. For each genomic sequence listed, the following oligonucleotides as detailed in the sequence listing may be used for a combined MSP-RealTime analysis:

SEQ ID NO: 16

left Primer: SEQ ID NOS:1160, 1163, 1166, 1169, 1171, 1172, 1173, 1174, 1175, 1178, 1179, 1183, 1184;

right Primer: SEQ ID NOS:1161, 1164, 1167, 1168, 1176, 1180, 1182, 1185;

Detection: SEQ ID NOS:1162, 1165, 1170, 1177, 1181.

SEQ ID NO: 4

left Primer: SEQ ID NOS:1186, 1190, 1191, 1192, 1195, 1196, 1199, 1200, 1203, 1205, 1206, 1208, 1209, 1211, 1213, 1214, 1216, 1219, 1221, 1223, 1225, 1230, 1234, 1240, 1241, 1242, 1245, 1247, 1249, 1252, 1257, 1258, 1260, 1264, 1265, 1266, 1267, 1271, 1273, 1274, 1275, 1277, 1280, 1281, 1282, 1287, 1288, 1289, 1293, 1294, 1295, 1296, 1299, 1301, 1304, 1306, 1308, 1310, 1312, 1320, 1321, 1323, 1324, 1327, 1329, 1331, 1333, 1336, 1339,

1340, 1341, 1348, 1350, 1353, 1357, 1359, 1361, 1366, 1367, 1371, 1374, 1375, 1376, 1379, 1381, 1384, 1385, 1386, 1389, 1390, 1393, 1394, 1398, 1402, 1405, 1408, 1413, 1416, 1419, 1420, 1422, 1423, 1429, 1431, 1435, 1436, 1437, 1440, 1442, 1444, 1446, 1447, 1449, 1451, 1454, 1456, 1459, 1460, 1461, 1464, 1466, 1468, 1471, 1473, 1474, 1479, 1480, 1481, 1482, 1483, 1488, 1490, 1493, 1494, 1495, 1505, 1506, 1508, 1510, 1513, 1515, 1519, 1522, 1523, 1524, 1526, 1527, 1528, 1531, 1532, 1533, 1535, 1536, 1539, 1540, 1542, 1544, 1548, 1551, 1553, 1554, 1555, 1558, 1559, 1564, 1567, 1569, 1572, 1573, 1576;

right Primer: SEQ ID NOS:1187, 1189, 1193, 1194, 1197, 1198, 1201, 1204, 1207, 1210, 1212, 1215, 1217, 1220, 1222, 1224, 1226, 1227, 1228, 1229, 1231, 1232, 1233, 1235, 1237, 1239, 1243, 1246, 1248, 1250, 1251, 1253, 1254, 1255, 1256, 1259, 1261, 1263, 1268, 1270, 1272, 1276, 1278, 1283, 1285, 1286, 1290, 1291, 1292, 1297, 1298, 1300, 1302, 1305, 1309, 1311, 1313, 1314, 1315, 1316, 1317, 1318, 1319, 1322, 1325, 1330, 1332, 1334, 1335, 1337, 1342, 1344, 1346, 1347, 1349, 1351, 1352, 1354, 1356, 1358, 1360, 1362, 1364, 1365, 1368, 1370, 1372, 1377, 1378, 1380, 1382, 1387, 1388, 1391, 1395, 1396, 1397, 1399, 1400, 1401, 1403, 1404, 1406, 1409, 1410, 1412, 1414, 1415, 1417, 1421, 1424, 1426, 1427, 1428, 1430, 1432, 1433, 1438, 1441, 1443, 1445, 1448, 1450, 1455, 1457, 1462, 1463, 1465, 1467, 1469, 1472, 1475, 1477, 1484, 1485, 1486, 1487, 1489, 1491, 1496, 1497, 1498, 1499, 1500, 1501, 1502, 1504, 1507, 1509, 1511, 1512, 1514, 1516, 1518, 1521, 1525, 1529, 1537, 1538; 1541, 1543, 1546, 1547, 1549, 1557, 1561, 1562, 1563, 1566, 1568, 1570, 1571, 1574, 1575;

Detection: SEQ ID NOS:1188, 1202, 1218, 1236, 1238, 1244, 1262, 1269, 1279, 1284, 1303, 1307, 1326, 1328, 1338, 1343, 1345, 1355, 1363, 1369, 1373, 1383, 1392, 1407, 1411, 1418, 1425, 1434, 1439, 1452, 1453, 1458, 1470, 1476, 1478, 1492, 1503, 1517, 1520, 1530, 1534, 1545, 1550, 1552, 1556, 1560, 1565.

SEQ ID NO: 27

left Primer: SEQ ID NOS:1577, 1580, 1583, 1587, 1588, 1592, 1594, 1595, 1596, 1603, 1604, 1605, 1607, 1608, 1609, 1611, 1612, 1618, 1624, 1626, 1627, 1628, 1629, 1630, 1632, 1633, 1635, 1638, 1643, 1644, 1645, 1649, 1653, 1654, 1657, 1660, 1665, 1666, 1668, 1671, 1676, 1681, 1686, 1693, 1702, 1703, 1704, 1706, 1709, 1712, 1713, 1715, 1716, 1720,

1729, 1730, 1734, 1735, 1736, 1740, 1743, 1745, 1753, 1756, 1759, 1761, 1764, 1765, 1766, 1769, 1771, 1772, 1776, 1779, 1780, 1783, 1787, 1788, 1790, 1792, 1794, 1796, 1797, 1798, 1801, 1803, 1806, 1812, 1816, 1818, 1819, 1820, 1824, 1827, 1828, 1829, 1830, 1832, 1833, 1839, 1841, 1842, 1844, 1845, 1848, 1853, 1854, 1858, 1861, 1863, 1864, 1867, 1870, 1871, 1874, 1878, 1881, 1883, 1885, 1886, 1887, 1892, 1895, 1899, 1902, 1906, 1911, 1919, 1922, 1926, 1927, 1928, 1929, 1931, 1932, 1934, 1938, 1941, 1946, 1947, 1948, 1950, 1951, 1953, 1956, 1957, 1958, 1964, 1965, 1967, 1971, 1979;

right Primer: SEQ ID NOS:1578, 1581, 1584, 1586, 1589, , 1949, 1952, 1954, 1955, 1959, 1960, 1961, 1962, 1963, 1966, 1968, 1969, 1970, 1972, 1973, 1974, 1975, 1976, 1977, 1978, 1980

Detection: SEQ ID NOS:1579, 1582, 1585, 1590, 1598, 1614, 1615, 1620, 1637, 1640, 1642, 1651, 1656, 1659, 1662, 1670, 1672, 1680, 1682, 1688, 1697, 1708, 1711, 1714, 1718, 1722, 1731, 1739, 1742, 1754, 1763, 1774, 1778, 1782, 1785, 1800, 1805, 1809, 1822, 1826, 1835, 1847, 1850, 1860, 1869, 1876, 1880, 1889, 1894, 1897, 1904, 1910, 1921, 1924, 1943, 1981,

SEQ ID NO: 32

left Primer: SEQ ID NOS:1982, 1988, 1989, 1992, 1996, 1997, 1998, 2001, 2002, 2005, 2006, 2009, 2011, 2013, 2014, 2015, 2016, 2021;

right Primer: SEQ ID NOS:1983, 1985, 1986, 1987, 1990, 1993, 1994, 1995, 1999, 2004, 2007, 2008, 2010, 2012, 2018, 2019, 2020, 2022, 2023

Detection: SEQ ID NOS:1984, 1991, 2000, 2003, 2017,

SEQ ID NO: 33

left Primer: SEQ ID NOS:2024, 2028, 2032, 2033, 2042, 2045, 2046, 2049, 2052, 2053, 2057, 2058, 2061, 2064, 2065, 2067, 2068, 2070, 2073, 2074, 2077, 2078, 2079, 2080, 2082, 2083, 2088, 2090, 2091, 2092, 2093, 2098

right Primer: SEQ ID NOS:2025, 2027, 2029, 2031, 2034, 2036, 2037, 2038, 2039, 2041, 2043, 2047, 2048, 2050, 2054, 2055, 2056, 2059, 2062, 2063, 2066, 2069, 2071, 2075, 2081, 2084, 2085, 2086, 2087, 2089, 2094, 2095, 2096, 2097;

Detection: SEQ ID NOS:026, 2030, 2035, 2040, 2044, 2051, 2060, 2072, 2076.

SEQ ID NO: 34

left Primer: SEQ ID NOS:2099, 2102, 2104, 2107, 2110, 2111, 2112, 2113, 2114, 2115, 2118, 2119, 2121, 2122, 2123, 2124, 2126, 2128, 2132, 2133, 2134, 2143, 2147, 2148, 2149, 2151, 2155;

right Primer: SEQ ID NOS:2100, 2105, 2108, 2116, 2125, 2127, 2129, 2130, 2131, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2144, 2146, 2150, 2152, 2153, 2154, 2156;

Detection: SEQ ID NOS:2101, 2103, 2106, 2109, 2117, 2120, 2145.

SEQ ID NO: 24

left Primer: SEQ ID NOS:2157, 2160, 2161, 2168, 2169, 2171, 2172, 2173, 2179, 2184, 2186, 2191, 2193, 2196, 2200, 2201, 2202, 2206, 2207, 2208, 2210, 2211, 2214, 2216, 2220, 2223, 2224, 2225, 2226, 2228, 2230, 2231, 2232, 2234, 2235, 2236, 2237, 2240, 2241, 2243, 2249, 2250, 2251, 2252, 2254, 2259, 2261, 2262, 2263, 2264, 2265, 2266, 2269, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2280, 2281, 2283, 2286, 2289, 2291, 2296, 2299, 2300, 2303, 2306, 2307, 2309, 2312, 2314, 2322, 2323, 2324, 2325, 2326, 2327, 2333;

right Primer: SEQ ID NOS:2158, 2162, 2164, 2165, 2166, 2167, 2170, 2174, 2176, 2177, 2178, 2180, 2181, 2182, 2183, 2185, 2187, 2189, 2190, 2192, 2194, 2195, 2197, 2198, 2199, 2203, 2205, 2209, 2212, 2215, 2217, 2218, 2219, 2221, 2227, 2229, 2233, 2238, 2242, 2244, 2245, 2246, 2247, 2248, 2255, 2257, 2258, 2260, 2267, 2270, 2278, 2282, 2284, 2287, 2290, 2292, 2294, 2295, 2297, 2301, 2304, 2308, 2310, 2313, 2316, 2317, 2318, 2319, 2320, 2321, 2328, 2329, 2330, 2331, 2332, 2334;

Detection: SEQ ID NOS:2159, 2163, 2175, 2188, 2204, 2213, 2222, 2239, 2253, 2256, 2268, 2279, 2285, 2288, 2293, 2298, 2302, 2305, 2311, 2315.

SEQ ID NO: 25

left Primer: SEQ ID NOS:2335, 2339, 2340, 2341, 2343, 2344, 2347, 2348, 2349, 2350, 2353, 2354, 2357, 2361, 2362, 2363, 2364, 2369, 2370, 2371, 2372, 2375, 2377, 2380;

right Primer: SEQ ID NOS:2336, 2338, 2342, 2345, 2351, 2355, 2358, 2360, 2365, 2367, 2368, 2373, 2376, 2378, 2379;

Detection: SEQ ID NOS:2337, 2346, 2352, 2356, 2359, 2366, 2374, 2381.

SEQ ID NO: 28

left Primer: SEQ ID NOS:2382, 2386, 2390, 2391, 2394, 2400, 2402, 2404, 2408, 2411, 2412, 2415, 2417, 2419, 2423, 2425, 2428, 2435, 2436, 2440, 2443, 2449, 2453, 2454, 2456, 2457, 2458, 2462, 2463, 2464, 2468, 2469, 2475, 2478, 2481, 2488, 2489, 2490, 2491, 2492, 2495, 2497, 2511, 2512, 2514, 2517, 2518, 2531, 2532, 2533, 2534, 2535, 2537, 2541, 2542, 2546, 2550, 2554, 2558, 2559;

right Primer: SEQ ID NOS:2383, 2385, 2387, 2389, 2392, 2393, 2395, 2396, 2397, 2398, 2399, 2401, 2403, 2405, 2407, 2409, 2413, 2414, 2416, 2418, 2420, 2421, 2422, 2424, 2426, 2429, 2431, 2432, 2433, 2434, 2437, 2438, 2439, 2441, 2442, 2444, 2445, 2446, 2447, 2448, 2450, 2452, 2455, 2459, 2460, 2461, 2466, 2467, 2470, 2472, 2473, 2474, 2476, 2479, 2480, 2482, 2483, 2484, 2485, 2486, 2487, 2493, 2494, 2496, 2498, 2499, 2500, 2501, 2502, 2503, 2504, 2505, 2506, 2507, 2508, 2509, 2510, 2513, 2515, 2516, 2519, 2520, 2521, 2522, 2523, 2525, 2526, 2527, 2528, 2530, 2536, 2538, 2540, 2543, 2544, 2545, 2547, 2548, 2549, 2551, 2553, 2555, 2556, 2557, 2560;

Detection: SEQ ID NOS:2384, 2388, 2406, 2410, 2427, 2430, 2451, 2465, 2471, 2477, 2524, 2529, 2539, 2552.

SEQ ID NO: 26

left Primer: SEQ ID NOS:2561, 2564, 2567, 2569, 2573, 2574, 2579, 2581, 2583, 2586, 2588, 2590, 2592, 2596, 2599, 2600, 2602, 2604, 2609, 2611, 2612, 2615, 2617, 2618, 2619, 2622, 2624, 2630, 2632, 2633, 2636, 2640, 2641, 2642, 2644, 2648, 2651, 2654, 2655, 2656, 2658, 2660, 2665, 2667, 2673, 2680, 2685, 2689, 2692, 2695, 2697, 2701, 2704, 2707, 2709, 2710, 2711, 2712, 2714, 2715, 2716, 2719, 2720, 2721, 2724, 2725, 2727, 2728, 2729, 2730, 2734, 2735, 2736, 2737, 2738, 2742, 2745, 2748, 2749, 2751, 2752, 2755, 2757, 2759, 2762, 2766, 2770, 2772, 2776, 2777, 2779, 2783, 2784, 2785, 2789, 2790, 2792, 2799, 2800,

2802, 2804, 2806, 2807, 2810, 2811, 2815, 2818, 2819, 2822, 2823, 2827, 2829, 2833, 2835, 2838, 2841, 2842, 2845, 2846, 2847, 2850, 2853, 2856, 2858, 2861, 2864, 2866, 2871, 2872, 2873, 2875, 2876, 2879, 2880, 2884, 2885, 2886, 2888, 2890, 2892, 2893, 2895, 2896, 2900, 2901, 2904, 2907, 2910, 2913, 2915, 2917, 2920, 2921, 2922, 2923, 2924, 2926, 2928, 2930, 2936, 2938, 2940, 2943, 2945, 2946, 2947, 2948, 2949, 2950, 2951, 2952, 2953, 2956, 2957, 2958, 2959;

right Primer: SEQ ID NOS:2562, 2565, 2568, 2570, 2572, 2575, 2577, 2580, 2582, 2584, 2587, 2589, 2591, 2593, 2594, 2595, 2597, 2601, 2603, 2605, 2607, 2608, 2610, 2613, 2620, 2623, 2625, 2626, 2627, 2628, 2629, 2631, 2634, 2637, 2638, 2639, 2643, 2645, 2647, 2649, 2652, 2657, 2659, 2661, 2662, 2663, 2664, 2666, 2668, 2669, 2670, 2672, 2674, 2676, 2677, 2681, 2683, 2684, 2686, 2688, 2690, 2693, 2694, 2696, 2698, 2699, 2700, 2702, 2705, 2708, 2713, 2717, 2722, 2726, 2731, 2733, 2739, 2741, 2743, 2744, 2746, 2747, 2750, 2753, 2758, 2760, 2763, 2765, 2767, 2769, 2771, 2773, 2774, 2775, 2780, 2781, 2782, 2786, 2788, 2791, 2793, 2795, 2796, 2797, 2798, 2801, 2803, 2805, 2808, 2812, 2813, 2814, 2816, 2817, 2820, 2821, 2824, 2825, 2826, 2828, 2830, 2832, 2834, 2836, 2839, 2840, 2843, 2848, 2851, 2854, 2855, 2859, 2860, 2863, 2865, 2867, 2869, 2877, 2881, 2883, 2887, 2889, 2894, 2897, 2899, 2902, 2905, 2908, 2909, 2911, 2914, 2916, 2918, 2925, 2927, 2929, 2931, 2932, 2933, 2934, 2935, 2937, 2939, 2942, 2944, 2954, 2955, 2960;

Detection: SEQ ID NOS:2563, 2566, 2571, 2576, 2578, 2585, 2598, 2606, 2614, 2616, 2621, 2635, 2646, 2650, 2653, 2671, 2675, 2678, 2679, 2682, 2687, 2691, 2703, 2706, 2718, 2723, 2732, 2740, 2754, 2756, 2761, 2764, 2768, 2778, 2787, 2794, 2809, 2831, 2837, 2844, 2849, 2852, 2857, 2862, 2868, 2870, 2874, 2878, 2882, 2891, 2898, 2903, 2906, 2912, 2919, 2941, 2961.

SEQ ID NO: 38

left Primer: SEQ ID NOS:2962, 2968;

right Primer: SEQ ID NOS:2963, 2965, 2966, 2967, 2969, 2971, 2972, 2973;

Detection: SEQ ID NOS:2964, 2970.

SEQ ID NO: 39

left Primer: SEQ ID NO:2974;

right Primer: SEQ ID NO:2975;

Detection: SEQ ID NO:2976.

SEQ ID NO: 40

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right Primer: SEQ ID NOS:2978, 2980, 2981, 2986, 2987, 2989, 2991, 2992, 2993, 2995, 2996, 2997, 2999, 3000, 3001, 3003, 3004, 3005, 3007, 3010, 3011, 3012, 3013, 3016, 3017, 3018, 3020, 3024, 3026, 3029, 3032, 3033, 3034;

Detection: SEQ ID NOS:979, 2990, 2994, 3008, 3014, 3021, 3027.

SEQ ID NO: 41

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Detection: SEQ ID NOS:3037, 3040, 3042, 3045, 3050, 3054, 3058, 3062, 3083, 3091, 3097, 3103, 3106, 3122, 3134, 3143, 3187, 3193, 3195, 3197, 3200, 3204, 3213, 3225, 3244, 3247, 3270, 3273, 3276, 3280.

SEQ ID NO: 5

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Detection: SEQ ID NOS:3285, 3290, 3301, 3313, 3317, 3322, 3325, 3329, 3332, 3334, 3337, 3342, 3350, 3354, 3357, 3361, 3365, 3368, 3376, 3381, 3385, 3388, 3398, 3411.

SEQ ID NO: 6

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Detection: SEQ ID NOS:3414, 3430, 3439, 3442, 3446, 3453, 3461, 3464, 3473, 3484, 3494, 3504.

SEQ ID NO: 8

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SEQ ID NO: 42

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3755, 3759, 3760, 3769, 3773, 3775, 3776, 3778, 3779, 3780, 3785, 3788, 3789, 3792, 3793, 3794, 3797, 3800, 3802, 3805, 3811, 3812, 3813, 3814, 3815, 3817, 3820, 3824, 3826, 3829, 3830, 3833, 3835, 3837, 3838, 3839, 3840, 3841, 3842, 3847, 3848, 3849, 3851, 3853, 3854, 3858, 3859, 3860, 3861, 3866, 3870, 3875, 3877, 3878, 3879, 3882, 3885, 3886, 3889, 3890, 3891, 3893, 3895, 3898, 3903, 3905, 3908, 3910, 3913, 3915, 3916, 3917, 3919, 3920, 3922, 3925, 3929, 3930, 3931, 3933;

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SEQ ID NO: 14

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Detection: SEQ ID NOS:3936, 3940, 3944, 3958, 3975.

SEQ ID NO: 15

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SEQ ID NO: 7

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SEQ ID NO: 1

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SEQ ID NO: 2

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Detection: SEQ ID NOS:4652, 4665, 4669.

SEQ ID NO: 45

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SEQ ID NO: 9

left Primer: SEQ ID NOS:7795, 7798, 7800, 7801, 7804, 7806, 7807, 7809, 7810, 7811, 7814, 7815, 7816, 7818, 7822, 7825, 7826, 7827, 7828, 7829, 7832, 7836, 7837, 7839, 7841, 7842, 7845, 7849, 7851, 7852, 7855, 7856, 7861, 7862, 7863, 7864, 7867, 7868, 7869, 7870, 7871;

right Primer: SEQ ID NOS:7796, 7799, 7802, 7805, 7808, 7812, 7817, 7819, 7821, 7824, 7830, 7833, 7835, 7838, 7840, 7843, 7846, 7848, 7850, 7853, 7857, 7858, 7859, 7860, 7865;

Detection: SEQ ID NOS:7797, 7803, 7813, 7820, 7823, 7831, 7834, 7844, 7847, 7854, 7866.

SEQ ID NO: 12

left Primer: SEQ ID NOS:7872, 7876, 7878, 7880, 7882, 7885, 7887, 7889, 7890, 7893, 7896, 7898, 7899, 7900, 7902, 7903, 7904, 7908, 7911, 7914, 7915, 7916, 7918, 7919, 7922, 7924, 7925, 7926, 7927, 7928, 7929, 7930, 7932, 7933, 7934, 7938, 7940, 7945, 7948;

right Primer: 7873, 7875, 7877, 7879, 7881, 7883, 7886, 7888, 7891, 7894, 7897, 7901, 7905, 7907, 7909, 7910, 7912, 7917, 7920, 7921, 7923, 7935, 7936, 7937, 7939, 7941, 7943, 7944, 7946;

Detection: 7874, 7884, 7892, 7895, 7906, 7913, 7931, 7942, 7947.

SEQ ID NO: 20

left Primer: SEQ ID NOS:7949, 7952, 7958, 7962, 7963, 7966, 7970, 7974, 7976, 7983, 7985, 7987, 7992, 7994, 7996, 7997, 7998, 8002, 8004, 8006, 8007, 8009, 8010, 8011, 8015, 8018, 8020, 8023, 8025, 8029, 8031, 8037, 8038, 8043, 8046, 8049, 8051, 8054, 8060, 8067, 8071, 8075, 8078, 8080, 8082, 8086, 8088, 8090, 8093, 8095, 8096, 8099, 8103, 8108, 8111, 8112, 8114, 8119, 8120, 8123, 8127, 8131, 8132, 8134, 8136, 8137, 8138, 8139, 8140, 8141, 8142, 8143, 8149, 8150, 8154, 8157, 8158, 8159, 8162, 8165, 8166, 8167, 8168, 8169, 8170, 8171, 8172, 8173, 8176;

right Primer: SEQ ID NOS:7950, 7953, 7955, 7956, 7959, 7961, 7964, 7967, 7969, 7971, 7972, 7973, 7977, 7979, 7980, 7981, 7982, 7984, 7986, 7988, 7990, 7991, 7995, 7999, 8000, 8001, 8003, 8005, 8008, 8012, 8014, 8016, 8017, 8019, 8021, 8022, 8024, 8026, 8027, 8028, 8030, 8032, 8033, 8034, 8035, 8039, 8040, 8041, 8042, 8044, 8047, 8050, 8052, 8055, 8056, 8057, 8058, 8059, 8061, 8063, 8064, 8065, 8066, 8068, 8070, 8072, 8074, 8076, 8083, 8085, 8089, 8091, 8098, 8100, 8102, 8104, 8105, 8106, 8107, 8109, 8115, 8117, 8118, 8122,

8124, 8125, 8126, 8128, 8129, 8130, 8133, 8135, 8144, 8146, 8148, 8151, 8153, 8155, 8156, 8160, 8163, 8174, 8175;

Detection: SEQ ID NOS:7951, 7954, 7957, 7960, 7965, 7968, 7975, 7978, 7989, 7993, 8013, 8036, 8045, 8048, 8053, 8062, 8069, 8073, 8077, 8079, 8081, 8084, 8087, 8092, 8094, 8097, 8101, 8110, 8113, 8116, 8121, 8145, 8147, 8152, 8161, 8164/

SEQ ID NO: 35

left Primer: SEQ ID NOS:8177, 8180, 8182, 8185, 8187, 8188, 8189, 8192, 8193, 8195, 8196, 8198, 8204, 8206, 8208, 8209, 8213, 8218, 8219, 8222, 8223, 8224, 8227, 8229, 8233, 8236, 8237, 8239, 8240, 8242, 8245, 8252, 8256, 8259, 8262, 8264, 8268, 8269, 8270, 8273, 8274, 8275, 8278, 8279, 8283, 8286, 8287, 8291, 8293, 8294, 8295, 8298, 8300, 8301, 8302, 8303, 8304, 8306, 8307, 8310, 8314, 8318, 8319, 8320, 8321, 8322, 8324, 8325, 8331, 8337, 8338, 8339, 8340, 8342, 8343, 8344, 8347, 8351, 8352, 8353, 8355, 8359, 8364, 8367, 8372, 8373, 8374, 8377, 8380, 8382, 8384, 8385, 8389, 8390, 8393, 8398, 8399, 8404, 8407, 8408, 8409, 8411, 8413, 8417, 8418, 8419, 8420, 8422, 8423, 8426, 8427, 8428, 8430, 8431, 8433;

right Primer: SEQ ID NOS:8178, 8181, 8183, 8186, 8190, 8194, 8197, 8199, 8201, 8202, 8203, 8205, 8207, 8210, 8211, 8212, 8214, 8216, 8217, 8220, 8221, 8225, 8230, 8232, 8234, 8238, 8241, 8243, 8246, 8248, 8249, 8250, 8253, 8255, 8257, 8260, 8261, 8263, 8265, 8267, 8271, 8276, 8280, 8282, 8284, 8285, 8288, 8290, 8292, 8296, 8297, 8299, 8305, 8308, 8309, 8311, 8313, 8315, 8316, 8317, 8323, 8326, 8328, 8329, 8330, 8332, 8333, 8334, 8335, 8336, 8341, 8345, 8346, 8349, 8350, 8354, 8356, 8358, 8360, 8362, 8363, 8365, 8368, 8370, 8375, 8378, 8379, 8381, 8383, 8386, 8387, 8388, 8391, 8394, 8396, 8397, 8400, 8401, 8402, 8403, 8405, 8406, 8410, 8412, 8414, 8415, 8421, 8424, 8429, 8432, 8434, 8435;

Detection: SEQ ID NOS:8179, 8184, 8191, 8200, 8215, 8226, 8228, 8231, 8235, 8244, 8247, 8251, 8254, 8258, 8266, 8272, 8277, 8281, 8289, 8312, 8327, 8348, 8357, 8361, 8366, 8369, 8371, 8376, 8392, 8395, 8416, 8425.

In an alternative most preferred embodiment of the method, the subsequent amplification of d) is carried out in the presence of *blocking oligonucleotides*, as described above. Said *blocking oligonucleotides* comprising a sequence having a length of at least 9 nucleotides which hybridizes to a pretreated nucleic acid sequence according to one of SEQ ID NOS:304 to SEQ ID NO:535 and sequences complementary thereto, wherein the base sequence of said oligomers comprises at least one CpG, TpG or CpA dinucleotide. Step e) of the method, namely the detection of the specific amplificates indicative of the methylation status of one or more CpG positions according to SEQ ID NOS:1 to SEQ ID NO:58 is carried out by means of real-time detection methods as described above.

Additional embodiments of the invention provide a method for the analysis of the methylation status of genomic DNA according to the invention (SEQ ID NOS:1 to SEQ ID NO:58, and complements thereof) without the need for pretreatment.

In the *first step* of such additional embodiments, the genomic DNA sample is isolated from tissue or cellular sources. Preferably, such sources include cell lines, histological slides, body fluids, or tissue embedded in paraffin. In the *second step*, the genomic DNA is extracted. Extraction may be by means that are standard to one skilled in the art, including but not limited to the use of detergent lysates, sonification and vortexing with glass beads. Once the nucleic acids have been extracted, the genomic double-stranded DNA is used in the analysis.

In a preferred embodiment, the DNA may be cleaved prior to the treatment, and this may be by any means standard in the state of the art, in particular with methylation-sensitive restriction endonucleases.

In the *third step*, the DNA is then digested with one or more methylation sensitive restriction enzymes. The digestion is carried out such that hydrolysis of the DNA at the restriction site is informative of the methylation status of a specific CpG dinucleotide.

In the *fourth step*, which is optional but a preferred embodiment, the restriction fragments are amplified. This is preferably carried out using a polymerase chain reaction, and said amplificates may carry suitable detectable labels as discussed above, namely fluorophore labels, radionuclides and mass labels.

In the *fifth step* the amplificates are detected. The detection may be by any means standard in the art, for example, but not limited to, gel electrophoresis analysis, hybridization analysis, incorporation of detectable tags within the PCR products, DNA array analysis, MALDI or ESI analysis.

In the final step the of the method the presence, absence or subclass of colon cell proliferative disorder is deduced based upon the methylation state of at least one CpG dinucleotide sequence of SEQ ID NOS:1 to SEQ ID NO:58 , or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotide sequences of SEQ ID NOS:1 to SEQ ID NO:58.

In a particularly preferred embodiment of the method the fourth step of the method comprises the use of at least one pair of MSP primers, and the use of hybridization probes for the detection of the subsequant amplificates by means of a real time assay.

Diagnostic and/or Prognostic Assays for colon cell proliferative disorders

The present invention enables diagnosis and/or prognosis of events which are disadvantageous to patients or individuals in which important genetic and/or epigenetic parameters within one or more of SEQ ID NOS:1 to SEQ ID NO:58 may be used as markers. Said parameters obtained by means of the present invention may be compared to another set of genetic and/or epigenetic parameters, the differences serving as the basis for a diagnosis and/or prognosis of events which are disadvantageous to patients or individuals.

Specifically, the present invention provides for diagnostic and/or prognostic cancer assays based on measurement of differential methylation of one or more CpG dinucleotide sequences of SEQ ID NOS:1 to SEQ ID NO:58, or of subregions thereof that comprise such a CpG dinucleotide sequence. Typically, such assays involve obtaining a tissue sample from a test tissue, performing an assay to measure the methylation status of at least one of one or more CpG dinucleotide sequences of SEQ ID NOS:1 to SEQ ID NO:58 derived from the tissue sample, relative to a control sample, or a known standard and making a diagnosis or prognosis based thereon.

In particular preferred embodiments, inventive oligomers are used to assess the CpG dinucleotide methylation status, such as those based on SEQ ID NOS:1 to SEQ ID NO:535, or arrays thereof, as well as in kits based thereon and useful for the diagnosis and/or prognosis of colon cell proliferative disorders.

Kits

Moreover, an additional aspect of the present invention is a kit comprising, for example: a bisulfite-containing reagent; a set of primer oligonucleotides containing at least two oligonucleotides whose sequences in each case correspond, are complementary, or hybridize under stringent or highly stringent conditions to a 16-base long segment of the sequences SEQ ID NOs:1 to SEQ ID NO:535; oligonucleotides and/or PNA-oligomers; as well as instructions for carrying out and evaluating the described method. In a further preferred embodiment, said kit may further comprise standard reagents for performing a CpG position-specific methylation analysis, wherein said analysis comprises one or more of the following techniques: MS-SNuPE, MSP, MethyLight ™, HeavyMethyl™ , COBRA, and nucleic acid sequencing. However, a kit along the lines of the present invention can also contain only part of the aforementioned components.

While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following example serves only to illustrate the invention and is not intended to limit the invention within the principles and scope of the broadest interpretations and equivalent configurations thereof.

EXAMPLES

Samples were received either as frozen tissue or extracted genomic DNA. DNA samples were extracted using lysis buffer from Qiagen and the Roche magnetic separation kit for genomic DNA isolation. DNA samples were also extracted using Qiagen Genomic Tip-100 columns, as well as the MagnaPure device and Roche reagents. All samples were quantitated using spectrophotometric or fluorometric techniques and on agarose gels for a subset of samples.

Bisulfite treatment and mPCR

Total genomic DNA of all samples was bisulfite treated converting unmethylated cytosines to uracil. Methylated cytosines remained conserved. Bisulfite treatment was performed with minor modifications according to the protocol described in Olek et al. (1996). In order to avoid processing all samples with the same biological background together resulting in a potential process-bias in the data later on, the samples were randomly grouped into processing batches. For bisulfite treatment we created batches of 50 samples randomized for sex, diagnosis, and tissue. Per DNA sample two independent bisulfite reactions were performed. After bisulfitation 10 ng of each DNA sample was used in subsequent mPCR reactions containing 6-8 primer pairs.

Each reaction contained the following:

0.4 mM each dNTPS

1 Unit Taq Polymerase

2.5 µl PCR buffer

3.5 mM MgCl2

80 nM Primerset (12-16 primers)

11.25 ng DNA (bisulfite treated)

Further details of the primers are shown in TABLE 1.

Forty cycles were carried out as follows: Denaturation at 95°C for 15 min, followed by annealing at 55°C for 45 sec., primer elongation at 65°C for 2 min. A final elongation at 65°C was carried out for 10 min.

1.1.2 Hybridization

All PCR products from each individual sample were then hybridised to glass slides carrying a pair of immobilised oligonucleotides for each CpG position under analysis. Each of these detection oligonucleotides was designed to hybridise to the bisulphite converted sequence around one CpG site which was either originally unmethylated (TG) or methylated (CG). See Table 2 for further details of all hybridisation oligonucleotides used (both

informative and non-informative.) Hybridisation conditions were selected to allow the detection of the single nucleotide differences between the TG and CG variants.

5 µl volume of each multiplex PCR product was diluted in 10 x Ssarc buffer (10 x Ssarc:230 ml 20 x SSC, 180 ml sodium lauroyl sarcosinate solution 20%, dilute to 1000 ml with dH2O). The reaction mixture was then hybridised to the detection oligonucleotides as follows. Denaturation at 95°C, cooling down to 10 °C, hybridisation at 42°C overnight followed by washing with 10 x Ssarc and dH2O at 42°C.

Further details of the hybridisation oligonucleotides are shown in TABLE 2.

Fluorescent signals from each hybridised oligonucleotide were detected using genepix scanner and software. Ratios for the two signals (from the CG oligonucleotide and the TG oligonucleotide used to analyse each CpG position) were calculated based on comparison of intensity of the fluorescent signals.

The samples were processed in batches of 80 samples randomized for sex, diagnosis, tissue, and bisulphite batch For each bisulfite treated DNA sample 2 hybridizations were performed. This means that for each sample a total number of 4 chips were processed.

<u>Data analysis methods</u>

Analysis of the chip data:

From raw hybridization intensities to methylation ratios;

The log methylation ratio (log(CG/TG)) at each CpG position is determined according to a standardized preprocessing pipeline that includes the following steps:

For each spot the median background pixel intensity is subtracted from the median foreground pixel intensity (this gives a good estimate of background corrected hybridization intensities):

For both CG and TG detection oligonucleotides of each CpG position the background corrected median of the 4 redundant spot intensities is taken;

For each chip and each CpG position the log(CG/TG) ratio is calculated;

For each sample the median of log(CG/TG) intensities over the redundant chip repetitions is taken.

This ratio has the property that the hybridization noise has approximately constant variance over the full range of possible methylation rates (Huber et al., 2002).

Principle Component Analysis

The principle component analysis (PCA) projects measurement vectors (*e.g.* chip data, methylation profiles on several CpGs etc.) onto a new coordinate system. The new coordinate axes are referred to as principal components. The first principal component spans the direction of the largest variance of the data. Subsequent components are ordered by decreasing variance and are orthogonal to each other. Different CpG positions contribute with different weights to the extension of the data cloud along different components. PCA is an unsupervised technique, *i.e.*, it does not take into account the labels of the data points (for further details see e.g. Ripley (1996)).

PCA is typically used to project high dimensional data (in our case methylation-array data) onto lower dimensional subspaces in order to visualize or extract features with high variance from the data. In the present report we use 2 dimensional projections for statistical quality control of the data. We investigate the effect of different process parameters on the chip data and exclude that changing process parameters cause large alterations in the measurement values.

A robust version of PCA is used to detect single outlier chips and exclude them from further analysis (Model et al., 2002).

Hypothesis testing

The main task is to identify markers that show significant differences in the average degree of methylation between two classes. A significant difference is detected when the nullhypothesis that the average methylation of the two classes is identical can be rejected with $p<0.05$. Because we apply this test to a whole set of potential markers we have to correct the p-values for multiple testing. This was done by applying the False Discovery Rate (FDR) method (Dudoit et al., 2002).

For testing the null hypothesis that the methylation levels in the two classes are identical we used the likelihood ratio test for logistic regression models (Venables and Ripley, 2002). The logistic regression model for a single marker is a linear combination of methylation measurements from all CpG positions in the respective genomic region of interest (ROI). A significant p-value for a marker means that this ROI has some systematic correlation to the question of interest as given by the two classes. However, at least formally it makes no statement about the actual predictive power of the marker.

Class prediction by supervised learning

In order to give a reliable estimate of how well the CpG ensemble of a selected marker can differentiate between different tissue classes we can determine its prediction accuracy by classification. For that purpose we calculate a methylation profile based prediction function using a certain set of tissue samples with their class label. This step is called training and it exploits the prior knowledge represented by the data labels. The prediction accuracy of that function is then tested by cross-validation or on a set of independent samples. As a method of choice, we use the support vector machine (SVM) algorithm (Duda (2001), Christiannini (2000)) to learn the prediction function. If not stated otherwise, for this report the risk associated with false positive or false negative classifications are set to be equal relative to the respective class sizes. It follows that the learning algorithm obtains a class prediction function with the objective to optimize accuracy on an independent test sample set. Therefore sensitivity and specificity of the resulting classifier can be expected to be approximately equal.

Estimating the performance of the tissue class prediction: Cross Validation

With limited sample size the cross-validation method provides an effective and reliable estimate for the prediction accuracy of a discriminator function and therefore in addition to the significance of the markers we provide cross-validation accuracy, sensitivity and specificity estimates. For each classification task, the samples were partitioned into 5 groups of approximately equal size. Then the learning algorithm was trained on 4 of these 5

sample groups. The predictor obtained by this method was then tested on the remaining group of independent test samples. The number of correct positive and negative classifications was counted over 5 runs for the learning algorithm for all possible choices of the independent test group without using any knowledge obtained from the previous runs. This procedure was repeated on up to 10 random permutations of the sample set. Note that the above-described cross-validation procedure evaluates accuracy, sensitivity and specificity using practically all possible combinations of training and independent test sets. It therefore gives a better estimate of the prediction performance than simply splitting the samples into one training sample set and one independent test set.

Results

Figures 1, 5, 9, 13, 16, 20, 24, 28 and 32 show ranked matrices of data obtained according to Examples 1 and 2 according to CpG methylation differences between the two classes of tissues, using an algorithim. The figures are shown in greyscale, wherein the most significant CpG positions are at the bottom of the matrix with significance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in grey, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. On the right side p values for the individual CpG positions are shown. The p values are the probabilities that the observed distribution occurred by chance in the data set.

Figures 2, 6, 10, 17, 21, 25, 29 and 33 show the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figures 3, 7, 11, 14, 18, 22, 26, 30 and 34 show ranked matrices of data obtained according to Examples 1 and 2 of the accuracy of the genewise linear support vector machine cross validations between the two classes of tissues, for the best performing markers. The figures are shown in greyscale, wherein the most significant CpG positions are at the bottom

of the matrix with significance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in grey, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. On the right side accuracy values for each individual genomic region of interest are shown.

Figures 4, 8, 12, 15, 18, 23, 27, 31 and 35 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification. The accuracy of each genomic region is represented as black squares, the specificity as unfilled diamonds, the sensitivity as unfilled squares. The accuracy as measured on the X-axis shows the fraction of correctly classified samples.

Colon Normal vs. Colorectal Cancer

In the first comparison 102 colorectal carcinoma samples were compared to 73 samples from normal colon, including colon polyps and colon inflammatory disorders.

Figure 1 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 2 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 3 shows the accuracy of the top 12 performing markers.

Figure 4 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-12, 15-20, 22, 25-36, 38-49, 51-58, and complements thereof.

Other Tissues vs. Colorectal Cancer

In this classification 73 colorectal carcinoma samples were compared to an 'other tissue' class consisting of 140 samples from non-colorectal carcinomas, peripheral blood

lymphocytes and other normal tissues of non-colorectal origin. These markers therefore enable the detection of colorectal carcinoma cells in′, for example, body fluids such as serum.

Figure 5 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 6 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 7 shows the accuracy of the top 12 performing markers.

Figure 8 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-23, 26-36, 38-43, 45-49, 51-58 and complements thereof.

Colon Normal and Other Tissue vs. Colon Cancer

In this classification 73 colorectal carcinoma samples were compared to 242 colon normal and 'other tissue' samples. The colon normal class consisted of healthy colon, colon polyps and inflammatory disorder colon tissue samples, the 'other tissues' consisted of samples from non-colorectal carcinomas, peripheral blood lymphocytes and other normal tissues of non-colorectal origin.

Figure 9 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 10 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 11 shows the accuracy of the top 12 performing markers.

Figure 12 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-3, 5-13, 15-23, 26-36, 38-43, 45-49, 51-58, and complements thereof.

Polyps vs. Colorectal Cancer

In this classification 73 colorectal carcinoma samples were compared to 51 colon polyp samples.

Figure 13 shows the multivariate test results of the top performing markers using the conservative Bonferroni corrected LogReg test.

Figure 14 shows the accuracy of the top 12 performing markers.

Figure 15 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:11, 25, 27, 38, 40, 45, 53, and complements thereof.

Colon normal vs. Colorectal cell proliferative disorder

In this calssification 124 colon cell proliferative disorder samples (consisting of colon polyps and colorectal carcinoma) were compared to 51 'normal colon' samples consisting of both healthy colon samples and colon tissue of inflammatory disorders.

Figure 16 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 17 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 18 shows the accuracy of the top 12 performing markers.

Figure 19 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-58, and complements thereof.

Colon Normal vs. Colorectal Cancer

In this classification 73 colorectal carcinoma samples were compared to 51 'normal colon' samples consisting of both healthy colon samples and colon tissue of inflammatory disorders.

Figure 20 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 21 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 22 shows the accuracy of the top 12 performing markers.

Figure 23 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-3, 5-23, 25-36, 38-49, 51-58, and complements thereof.

<u>Colon Normal and Other Tissues vs. Colon Cell Proliferative Disorder</u>

In this classification 124 colon cell proliferative disorder samples (consisting of colon polyps and colorectal carcinoma) were compared to a class consisting of 'colon normal' and 'other tissue' samples. The colon normal samples consisted of both healthy colon samples and colon tissue of inflammatory disorders, the 'other tissue' samples consisted of samples from non-colorectal carcinomas, peripheral blood lymphocytes and other normal tissues of non-colorectal origin.

Figure 24 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 25 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 26 shows the accuracy of the top 12 performing markers.

Figure 27 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-36, 38-43, 45-58, and complements thereof.

Other Tissue vs. Colon Tissue

The following comparison was carried out in order to identify markers capable of discerning elevated levels of free floating colon DNA, especially in bodily fluids as a marker of tumor progression. In this classification the 'colon tissue' class consisted of samples from colorectal carcinoma, colon polyps, colon tissue of inflammatory disorders and healthy colon tissue. The 'other tissue' class consisted of samples from non-colorectal carcinomas, peripheral blood lymphocytes and other normal tissues of non-colorectal origin.

Figure 28 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 29 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 30 shows the accuracy of the top 12 performing markers.

Figure 31 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-58, and complements thereof.

Normal Tissue vs. Cell Proliferative Disorder Tissue

In this classification the gene panel was assessed for its ability to accurately discriminate cell proliferative disorder samples from both colorectal carcinoma, colon polyps and non-colon origin cancers from 'normal tissues', namely healthy colon samples, colon tissue of inflammatory disorders, peripheral blood lymphocytes, other normal tissues of non-colorectal origin.

Figure 32 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 33 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 34 shows the accuracy of the top 12 performing markers.

Figure 35 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-36, 38-43, 45-49, 51-58, and complements thereof.

The following examples describe the analysis of the methylation status of the genomic sequences SEQ ID NO:35, SEQ ID NO:34 , SEQ ID NO:39, SEQ ID NO:29 in healthy and sick colon cell proliferative disorder samples. The initial link between said genes and colon cell proliferative disorders was initially carried by means of hybridisation analysis as described in EXAMPLE 1. The sequences were then selected from the larger set of genes analysed in said example, and the correlation between methylation status and colon cell proliferative disorder states was validated by analysis of samples using other methylation analysis techniques, namely the MSP- MethyLight ™ and HeavyMethyl ™ MethyLight ™ assays. Please note that the term 'MethyLight ™' is used to describe real time PCR analysis of bisulfite treated DNA using probes of both the Taqman ™ (single probe) and Lightcycler ™ (dual probe) technologies.

EXAMPLE 2

Analysis of methylation within colon cancer using an MSP- MethyLight assay (SEQ ID NO 35) DNA was extracted from 33 colon adenocarcinoma samples and 43 colon normal adjacent tissues using a Qiagen extraction kit. The DNA from each sample was treated using a bisulfite solution (hydrogen sulfite, disulfite) according to the agarose-bead method (Olek et al 1996). The treatment is such that all non methylated cytosines within the sample are converted to thymidine. Conversely, 5-methylated cytosines within the sample remain unmodified.

The methylation status was determined with a MSP-MethyLight assay designed for the CpG island of interest and a control fragment from the *beta* actin gene (Eads et al., 2001). The CpG island assay covers CpG sites in both the primers and the Taqman style probe, while the control gene does not. The control gene is used as a measure of total DNA concentration, and the CpG island assay (methylation assay) determines the methylation levels at that site.

Methods: The SEQ ID NO 35 gene CpG island assay was performed using the following primers and probes:Forward Primer: CGGAGGGTACGGAGATTACG (SEQ ID NO: 8436);Reverse Primer: CGACGACGCGCGAAA (SEQ ID NO:8437); and Probe: CGAAACCCTAAATATCCCGAATAACGCCG (SEQ ID NO: 8438).The corresponding control assay was performed using the following primers and probes:Primer: TGGTGATGGAGGAGGTTTAGTAAGT (SEQ ID NO: 8439);Primer: AACCAATAAAACCTACTCCTCCCTTAA (SEQ ID NO: 8440); and Probe: ACCACCACCCAACACACAATAACAAACACA (SEQ ID NO:8441)The reactions were run in triplicate on each DNA sample with the following assay conditions:*Reaction solution*: (900 nM primers; 300 nM probe; 3.5 mM Magnesium Chloride; 1 unit of taq polymerase; 200 μM dNTPs; 7μl of DNA, in a final reaction volume of 20 μl);*Cycling conditions*: (95°C for 10 minutes; then 50 cycles of: 95°C for 15 seconds; 60°C for 1 minute).

The data was analysed using a PMR calculation previously described in the literature (Eads et al 2001).*Results*. The mean PMR for normal samples was 0.15, with a standard deviation of 0.18. The mean PMR for tumour samples was 17.98, with a standard deviation of 18.18. The overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.00000312). The results are shown in Figure 36. A Receiver Operating Characteristic curve (ROC curve) of the assay was also determined. A ROC is a plot of the true positive rate against the false positive rate for the different possible cutpoints of a diagnostic test. It shows the tradeoff between sensitivity and specificity depending on the selected cutpoint (any increase in sensitivity will be accompanied by a decrease in specificity). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test (the larger the area the better, optimum is 1, a random test would have a ROC curve lying on the diagonal with an area of 0.5; for reference: J.P. Egan. Signal Detection

Theory and ROC Analysis, Academic Press, New York, 1975). The AUC for the MSP-MethylLight™-Assay is: 0.94 (Figure 37).

EXAMPLE 3

Methylation of SEQ ID NO 35 within colon cancer was analysed using a HeavyMethyl MethyLight™ assay. The same DNA samples were also used to analyse methylation of the CpG island with a HeavyMethyl MethyLight™ (or HM MethyLight™) assay, also referred to as the HeavyMethyl™ assay. The methylation status was determined with a HM MethyLight™ assay designed for the CpG island of interest and the same control gene assay described above. The CpG island assay covers CpG sites in both the blockers and the Taqman™ style probe, while the control gene does not.

Methods. The CpG island assay (methylation assay) was performed using the following primers and probes:

Forward Primer: GGTGATTGTTTATTGTTATGGTTTG (SEQ ID NO:8442)

Reverse Primer: CCCCTCAACCTAAAAACTACAAC (SEQ ID NO:8443)

Forward Blocker: GTTATGGTTTGTGATTTTGTGTGGG (SEQ ID NO:8444)

Reverse Blocker: AAACTACAACCACTCAAATCAACCCA (SEQ ID NO:8445)

Probe: AAAATTACGACGACGCCACCCGAAA (SEQ ID NO:8446)

The reactions were each run in triplicate on each DNA sample with the following assay conditions:

Reaction solution: (400 nM primers; 400 nM probe; 10μM both blockers; 3.5 mM magnesium chloride; 1x ABI Taqman buffer; 1 unit of ABI TaqGold polymerase; 200μM dNTPs; and 7μl of DNA, in a final reaction volume of 20 μl);

Cycling conditions: (95□C for 10 minutes); (95°□C for 15 seconds, 64°C for 1 minute (2 cycles)); (95°C for 15 seconds, 62°C for 1 minute (2 cycles); (95°C for 15 seconds, 60°C for 1 minute (2 cycles)); and (95°C for 15 seconds, 58°C for 1 minute, 60°C for 40 seconds (41 cycles)).

Results. The mean PMR for normal samples was 1.12 with a standard deviation of 1.45. The mean PMR for tumour samples was 38.23 with a standard deviation of 33.22. The

overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.000000326). The results are shown in Figure 36.

A ROC curve of the assay was also determined. The AUC for the MSP-Methyl-Light-Assay is 0.91 (Figure 38)

The assay was tested on an additional set of colon samples (25 adenocarcinoma, 33 normals, and 13 adenomas). The results showed a significant difference again (Figure 39). The ROC are shown in Figure 40-42.

The MSP and HeavyMethyl variants of the MethyLight assay were determined to be equivalent for the analysis of methylation in SEQ ID NO 35. Figure 48 shows the regression plot of the percentage methylation detected in each sample using the two methods.

EXAMPLE 4

The SEQ ID NO 35 -HeavyMethyl-MethyLight-assay was also tested against a panel of other tissues (Figure 43). Besides the colon cancer samples only one of the two breast cancer tissues were methylated. However, on a panel of 21 additional breast tumours (different stages), only one was methylated (Figure 44). So the marker is specific for colon tumour samples. All primers, probes, blockers and reaction conditions were identical to those used in the analysis of the colon cancer samples (Example 3).

EXAMPLE 5

Twelve of the colon tissues analysed by real-time PCR also had paired serum taken before surgery. We extracted DNA from 1 ml of that serum using a Qiagen UltraSens□ DNA extraction kit, bisulfite treated the DNA sample, and ran the SEQ ID NO 35 -HeavyMethyl-MethyLight-assay on those samples. The control gene did not amplify for three of the cancer serum samples and three of the normal serum samples, so we can conclude that the sample preparation did not work in these cases. In the other cases, there was evidence of higher methylation in the cancer samples than the normal samples (Figure 45).

EXAMPLE 6

Analysis of methylation within colon cancer using a SEQ ID NO:34 -MSP- MethyLight Assay. The colon cancer samples described in Example 2 were also analysed using a SEQ ID NO:34 -MSP- MethyLight Assay with a Taqman® style probe. The sample preparation was carried out as described above (Example 1). The assay was performed using the following primers and probes:

Forward Primer: TGGGATTAAGATTTTCGGTTAGTTTC (SEQ ID NO: 8447)

Reverse Primer: CACTACAACGCTACGCGACTAAA (SEQ ID NO:8448)

Probe: TCGACGTTACCCAAACGAATCACATAAAAAAC (SEQ ID NO: 8449)

The corresponding control assay was performed as described above (EXAMPLE 2)

The reactions were run in triplicate on each DNA sample with the following assay conditions:

Reaction solution: (900 nM primers; 300 nM probe; 3.5 mM magnesium chloride; 1 units of taq polymerase; 200μM dNTPs, 5μM blocker; and 7μl of DNA, in a final reaction volume of 20 μl);

Cycling conditions: 95°C for 10 minutes; (95°C for 15 seconds, 60°C for 1 minute) 50 cycles

The data was analysed using a PMR calculation previously described in the literature (Eads et al 2001).

Results. The results are shown in Figure 36. The mean PMR for normal samples was 3.93, with a standard deviation of 3.57. The mean PMR for tumour samples was 23.06, with a standard deviation of 20.23 The overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.000003063). The ROC curve of the assay is shown in Figure 46. The AUC is 0.84.

This was further confirmed using a SEQ ID NO:34 -HeavyMethyl MethyLight™ assay, using dual Lightcycler probes.

Methods. The CpG island assay (methylation assay) was performed using the following primers and probes:

Forward Primer: TGGATAGGAGTTGGGATTAAGATTTT (SEQ ID NO:8450)

Reverse Primer: CTTATTACAATTTAAAAAAAAAATTCACTACAA (SEQ ID NO: 8451);

Blocker: AAATTCACTACAACACTACACAACTAAATTCAACATTAC (SEQ ID NO:8452);

Probe: TTTTCGTATTTTTTTTCGGGTTATTACGTTTT-Fluor (SEQ ID NO: 8453);

Probe: LC640-ATGTGATTCGTTTGGGTAACGTCGA-Phos (SEQ ID NO:8454.

The reactions were each run in triplicate on each DNA sample with the following assay conditions:

Reaction conditions: 500nM primers; 10uM blocker; 250nM probes; 4mM Magnesium Chloride

Cycling profile: 95 degree denaturation for 10 minutes; 50 cycles: 95 degrees 10 seconds, 57 degrees 30 seconds, 72 degrees 20 seconds

EXAMPLE 7

Analysis of methylation within colon cancer using a SEQ ID NO:29 -MSP-MethyLight™ Assay. The colon cancer samples described in Example 2 were also analysed using a SEQ ID NO:29 -MSP- MethyLight™ Assay with a Taqman® style probe. The sample preparation was carried out as described above (Example 2). The assay was performed using the following primers and probes:

Forward Primer: TTTTTTTTTCGGACGTCGTTG (SEQ ID NO 8457)

Reverse Primer: CCTCTACATACGCCGCGAAT (SEQ ID NO:8458)

Probe: AATTACCGAAAACATCGACCGA (SEQ ID NO:8459)

The reactions were run in triplicate on each DNA sample with the following assay conditions:

Reaction solution: (900 nM primers; 300 nM probe; 3.5 mM magnesium chloride; 1 units of taq polymerase; 200μM dNTPs, 5μM blocker; and 7μl of DNA, in a final reaction volume of 20 μl);

Cycling conditions: 95°C for 10 minutes; (95°C for 15 seconds, 60°C for 1 minute) 50 cycles

The corresponding control assay was performed as described above (EXAMPLE 2).

The data was analysed using a PMR calculation previously described in the literature (Eads et al 2001).

Results. The results are shown in Figure 36. The mean PMR for normal samples was 3.04, with a standard deviation of 4.21. The mean PMR for tumour samples was 21.38, with a standard deviation of 24.08 The overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.0000101973). The ROC curve of the assay is shown in Figure 47. The AUC is 0.80.

This was further confirmed using a SEQ ID NO:29 -HeavyMethyl MethyLight assay (using dual labeled Lightcycler probes.

Methods. The CpG island assay (methylation assay) was performed using the following primers and probes:

Forward Primer: GTAGGGTTATTGTTTGGGTTAATAAAT (SEQ ID NO: 8458)

Reverse Primer: TAAAAAAAAAAAAAAAACTCCTCTACATAC (SEQ ID NO: 8459)

Blocker: AACTCCTCTACATACACCACAAATAAATT (SEQ ID NO: 8460)

Probe: CGAAAACATCGACCGAACAACG-Fluor (SEQ ID NO: 8461)

Probe: LC640-GTCCGAAAAAAAAAAAACGAACTCC-Phos (SEQ ID NO: 8462)

The reactions were each run in triplicate on each DNA sample with the following assay conditions:

Reaction conditions: Forward primer: 600nM; Reverse primer: 300nM; Blocker: 10uM; Probes: 500nM; Taq polymerase: 0.1 U/ul; dNTPs: 0.2mM each; Magnesium Chloride: 4mM; BSA: 0.25 mg/ml; Roche buffer with no MgCl: 1x

Cycling conditions: 95-degree denaturation for 10 minutes; 50 cycles: 95-degrees for 10 seconds, 57-degrees for 25 seconds, 72 degrees for 10 seconds

EXAMPLE 8

Analysis of methylation within colon cancer using a SEQ ID NO:29 -MSP-MethyLight™ Assay. An additional assay for SEQ ID NO:29 was tested on colon samples.

The assay was tested on two sets of tissues, each with 12 colon adenocarcinomas and 12 normal adjacent tissue samples.

The sample preparation was carried out as described above (Example 2) The assay was performed using the following primers and probes:

Forward Primer: GGACGTTTTTATCGAAGGCG (SEQ ID NO: 8463)

Reverse Primer: GCCACCCAACCGCGA (SEQ ID NO:8464)

Probe: ACCCGAAATCACGCGCGAAAAA (SEQ ID NO:8465)

The reactions were run in triplicate on each DNA sample with the following assay conditions:

Reaction solution: (900 nM primers; 300 nM probe; 3.5 mM magnesium chloride; 1 units of taq polymerase; 200μM dNTPs, 5μM blocker; and 7μl of DNA, in a final reaction volume of 20 μl);

Cycling conditions: 95°C for 10 minutes; (95°C for 15 seconds, 60°C for 1 minute) 50 cycles. The corresponding control assay was performed as described above (Example 2)

The data was analysed using a PMR calculation previously described in the literature (Eads et al 2001). In both cases, SEQ ID NO:29 was significantly more methylated in the cancer samples The ROC curves of the assays are shown in Figures 49 and 50. The AUC are 0.93 and 1.

EXAMPLE 9

Analysis of methylation within colon cancer using a SEQ ID NO 39 -MSP-MethyLight Assay

The colon cancer samples described in Example 2 were also analysed using a SEQ ID NO 39 -MSP- MethyLight Assay. The sample preparation was carried out as described above (EXAMPLE 2) The assay was performed using the following primers and probes:

Forward Primer: GACGGATTTTTTTTAACGTTTTTC (SEQ ID NO:8466)

Reverse Primer: AAATAAAATACCACCTCCGCGA (SEQ ID NO:8467)

Probe: GCTCCTCGCGAAATACTCACCCCG (SEQ ID NO:8468)

The reactions were run in triplicate on each DNA sample with the following assay conditions:

Reaction solution: (900 nM primers; 300 nM probe; 3.5 mM magnesium chloride; 1 units of taq polymerase; 200μM dNTPs, 5μM blocker; and 7μl of DNA, in a final reaction volume of 20 μl);

Cycling conditions: 95°C for 10 minutes; (95°C for 15 seconds, 60°C for 1 minute) 50 cycles.

The corresponding control assay was performed as described above (EXAMPLE 2).

The data was analysed using a PMR calculation previously described in the literature (Eads et al 2001).

Results. The results are shown in Figure 36. The mean PMR for normal samples was 2.25, with a standard deviation of 2.42. The mean PMR for tumour samples was 25.67, with a standard deviation of 17.57 The overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.00000000118). The ROC curve of the assay is shown in Figure 52. The AUC is 0.94

This was further confirmed using a SEQ ID NO:39 -HeavyMethyl MethyLight assay, using dual Lightcycler probes using Lightcycler style dual probe technology.

Methods. The CpG island assay (methylation assay) was performed using the following primers and probes:

Forward Primer: GTTAGTTAGTTAATTTTTAAATAGATTAGTAG (SEQ ID NO:8469)

Reverse Primer: CAAAAAAACAAATAAAATACCACCTCC (SEQ ID NO:8470)

Blocker: CCTCCACAAAACTCACTCCTCACAAAATAC (SEQ ID NO:8471)

Probe: red640 TTTCGTTTTGTATGGTAGATACGGGGTGA- phosphate (SEQ ID NO: 8473)

Probe: ATTAATGGTTTTATAAGACGGATTTTTTTTAACGT- fluorescine (SEQ ID NO:8474)

The reactions were each run in triplicate on each DNA sample with the following assay conditions:

Reaction conditions: Forward primer: 600nM; Reverse primer: 300nM; Blocker: 10uM; Probes: 500nM; Taq polymerase: 0.1 U/ul; dNTPs: 0.2mM each; Magnesium Chloride: 4mM; BSA: 0.25 mg/ml; Roche buffer with no MgCl: 1x

Cycling conditions: 95-degree denaturation for 10 minutes; 50 cycles: 95-degrees for 10 seconds, 57-degrees for 25 seconds, 72 degrees for 10 seconds.

TABLES

TABLE 1.

No:	***Gene:***	***Primer:***	***Amplificate Length:***
(SEQ ID NO: 16)	TTGGAGTTAAAG TATTTGGTAAGA (SEQ ID NO: 536) AAAACCACCTTC AAACCC (SEQ ID NO: 537)		442
(SEQ ID NO: 4)	ATCCTCCACACT CTTCCTCTAT (SEQ ID NO: 538) GAAATTAGGTTT GGTTTTGTTT (SEQ ID NO: 539)		140
(SEQ ID NO: 4)	GAGATTTTGGGA GGGGTAG (SEQ ID NO: 540) AACTCTATCCTT TTCCCTCTTC (SEQ ID NO: 541)		486
(SEQ ID NO: 56)	TGTTGGTTGTTG TTGTTGTT (SEQ ID NO: 542) CTTTCTACCCAT CCCAAAA (SEQ ID NO: 543)		319
(SEQ ID NO: 27)	TAAGTGATAAAG GAAGGAAGGA (SEQ ID NO: 544) CCTTCAAACCCC		243

No:	*Gene:*	*Primer:*	*Amplificate Length:*
	AAACAA (SEQ ID NO: 545)		
(SEQ ID NO: 31)	TTGTTGTTTTTAG GGGTTTTGG (SEQ ID NO: 546) TCCTTCCCATTCT CCAAATATC (SEQ ID NO: 547)		947
(SEQ ID NO: 32)	TCAACTACCATC AACTTCCTTA (SEQ ID NO: 548) AATTTATTTTTA GTGTTGTAGTGG G (SEQ ID NO: 549)		491
(SEQ ID NO: 33)	GAAAGGAGAGG TTAAAGGTTG (SEQ ID NO: 550) AACTCACTTAAC TCCAATCCC (SEQ ID NO: 551)		696
(SEQ ID NO: 34)	GGATAGGAGTTG GGATTAAGAT (SEQ ID NO: 552) AAATCTTTTTCA ACACCAAAAT (SEQ ID NO: 553)		414
(SEQ ID NO: 24)	TCCAATAAACAC AAACCTAAATC (SEQ ID NO: 554) ATATGGGATTGA TGGAAGATAG (SEQ ID NO: 555)		471
(SEQ ID NO: 35)	GGAAGAGGTGA TTAAATGGAT (SEQ ID NO: 556) CCCAAAAATCAA ACAACAA (SEQ ID NO: 557)		226
(SEQ ID NO: 57)	ATTTGGGAAAGA GGGAAAG (SEQ ID NO: 558) TAAAAACTCTAA ACCCCATCC (SEQ ID NO: 559)		300
(SEQ ID NO: 25)	CCCTACCCACCA ATATACC (SEQ ID NO: 560) AGATTTGGGGAA		278

No:	*Gene:*	*Primer:*	*Amplificate Length:*
	GAAGTTGTA (SEQ ID NO: 561)		
(SEQ ID NO: 36)	TAAAAGAAGGA TTTTTGATTGG (SEQ ID NO: 562) CATCTTATTTAC CTCCCTCCC (SEQ ID NO: 563)		528
(SEQ ID NO: 28)	TTTTAGATTGAG GTTTTAGGGT (SEQ ID NO: 564) ATCCATTCTACC TCCTTTTTCT (SEQ ID NO: 565)		497
(SEQ ID NO: 37)	GTAATTTGAAGA AAGTTGAGGG (SEQ ID NO: 566) CCAACAACTAAA CAAAACCTCT (SEQ ID NO: 567)		296
(SEQ ID NO: 26)	AGTAAATAGTGG GTGAGTTATGAA (SEQ ID NO: 568) GAAAAACCTCTA AAAACTACTCTC C (SEQ ID NO: 569)		607
(SEQ ID NO: 38)	GTTAGTATGTTT GGGGGTAAAT (SEQ ID NO: 570) ATAAATAACACC TTCCACCCTA (SEQ ID NO: 571)		435
(SEQ ID NO: 39)	TTTGTATTAGGT TGGAAGTGGT (SEQ ID NO: 572) CCCAAATAAATC AACAACAACA (SEQ ID NO: 573)		286
(SEQ ID NO: 29)	TTGTTTGGGTTA ATAAATGGA (SEQ ID NO: 574) CTTCTCTCTTCTC CCCTCTC (SEQ ID NO: 575)		295
(SEQ ID NO: 40)	AATATAGGGAG GTTTAGGGTTT (SEQ ID NO: 576) TAACCATACATT		424

No:	Gene:	Primer:	Amplificate Length:
	TCTCATCCAA (SEQ ID NO: 577)		
(SEQ ID NO: 41)	TTTTGGGGAATA TAGGGTTT (SEQ ID NO: 578) TTCTCACATTTCT AACCACTTCT (SEQ ID NO: 579)		425
(SEQ ID NO: 5)	CTCCTCCTTCCA ACAAAAA (SEQ ID NO: 580) GTTTAGAGGTTT TGGGATGATT (SEQ ID NO: 581)		487
(SEQ ID NO: 5)	TGAATAGGGTGA TATTTTAGTTAG G (SEQ ID NO: 582) ATAAATCATCCC AAAACCTCTA (SEQ ID NO: 583)		497
(SEQ ID NO: 6)	ATTTGGTTATTG GTTGAAGGTA (SEQ ID NO: 584) AATTTTTAATTT CTCAACACCTCT (SEQ ID NO: 585)		361
(SEQ ID NO: 6)	GAAGAGGTGTTG AGAAATTAAAA (SEQ ID NO: 586) CCCACCCTAACT TACCATAAA (SEQ ID NO: 587)		462
(SEQ ID NO: 8)	CAATTCCCCTTA TTTCTCTAAA (SEQ ID NO: 588) AATTAGTTATGG TGTTGTGGGA (SEQ ID NO: 589)		339
(SEQ ID NO: 8)	TTCTATTAAAAC CCAACTCCTC (SEQ ID NO: 590) ATAAGGGGAATT GTTGTAGGTT (SEQ ID NO: 591)		395
(SEQ ID NO: 42)	TACCATTCTTTC CTAAACATCC (SEQ ID NO: 592) GGGTTGGTGGAG		148

No:	Gene:	Primer:	Amplificate Length:
	AGGTAG (SEQ ID NO: 593)		
(SEQ ID NO: 14)	GAAGATGAGAGGAGGTTGAGA (SEQ ID NO: 594) CCACACCACCTACTACAAAAT (SEQ ID NO: 595)		500
(SEQ ID NO: 15)	AACAAACCTCCTCCAAATTC (SEQ ID NO: 596) TGTTGGTAGGTATTGGTGATT (SEQ ID NO: 597)		365
(SEQ ID NO: 15)	TCCCCACTTAAAATAAACAAAT (SEQ ID NO: 598) GTGAATTTGGAGGAGGTTT (SEQ ID NO: 599)		375
(SEQ ID NO: 7)	GGGGTTGATATTGTTTTTAGAG (SEQ ID NO: 600) CCCCTCCTTCCTTAAATCT (SEQ ID NO: 601)		328
(SEQ ID NO: 44)	TTTTAGAAGGGGTTGGTTTAG (SEQ ID NO: 602) ACTACCTAACTCTCCCCACAA (SEQ ID NO: 603)		343
(SEQ ID NO: 44)	TTGTGGGGAGAGTTAGGTAGT (SEQ ID NO: 604) TAACCCAAATATCATAAAACCC (SEQ ID NO: 605)		411
(SEQ ID NO: 1)	AGATGGATATTTTGTTGGTGTT (SEQ ID NO: 606) TACACAATTATACCTTTCAAACAAT (SEQ ID NO: 607)		250
(SEQ ID NO: 1)	CCATACAAATATCCTAAATAAAACC (SEQ ID NO: 608)		482

No:	Gene:	Primer:	Amplificate Length:
	TTGTTGGAAGAA TTGATAGTGTT (SEQ ID NO: 609)		
(SEQ ID NO: 1)	AGGGAGTTAAGT AAGGGGTTAG (SEQ ID NO: 610) AACACCAACAA AATATCCATCT (SEQ ID NO: 611)		442
(SEQ ID NO: 2)	TGGAATTTTAGG GTTAGTAGGG (SEQ ID NO: 612) CAAATAAACCAA ACCACTATCA (SEQ ID NO: 613)		499
(SEQ ID NO: 2)	TTGGGATTTTTA GGTGGTTT (SEQ ID NO: 614) TTCACTTTCCCT ACTAACCCTA (SEQ ID NO: 615)		492
(SEQ ID NO: 45)	TGGGTTATTTGG TGAGTATTGT (SEQ ID NO: 616) CTTACCCCCACC CAACTA (SEQ ID NO: 617)		461
(SEQ ID NO: 46)	TTCACATTACAT TAACCCATTTA (SEQ ID NO: 618) TTGGAGTTGTTA GGAGAAAAGT (SEQ ID NO: 619)		452
(SEQ ID NO: 10)	CCTTCCTTAAAA ACCTCAAAAC (SEQ ID NO: 620) GTAAAGAATGGT AGAGGATAGGA T (SEQ ID NO: 621)		436
(SEQ ID NO: 10)	CTTACTACCCAA CCTCTTTCAC (SEQ ID NO: 622) TGGAAGGATAG AGAATTTTGTT (SEQ ID NO: 623)		444
(SEQ ID NO:	ATCCCATCTCTC AACTCCTACT (SEQ ID NO: 624)		452

No:	*Gene:*	*Primer:*	*Amplificate Length:*
11)	TGATTTATTTTG ATGTGTGGTT (SEQ ID NO: 625)		
(SEQ ID NO: 11)	TATTTAAGGATT TTGGAAGGAG (SEQ ID NO: 626) TCATCTCATTTT ATCTCTACAACC (SEQ ID NO: 627)		349
(SEQ ID NO: 13)	AACAAATTCCCA ACACACC (SEQ ID NO: 628) TTTTGGAAGATG GTTTATTTTT (SEQ ID NO: 629)		476
(SEQ ID NO: 13)	TTTTTAATATGG AGGTAAGGGA (SEQ ID NO: 630) AAATTCCCAACA CACCAAC (SEQ ID NO: 631)		279
(SEQ ID NO: 3)	CTTCTCCAAAAT CAACCAACT (SEQ ID NO: 632) TTTGTGTTATTA GTAGGTGAGAG G (SEQ ID NO: 633)		254
(SEQ ID NO: 3)	TTAGAAGTTGGA GGGTGAAAT (SEQ ID NO: 634) CTTCCTACCTTA AACCCTTACC (SEQ ID NO: 635)		450
(SEQ ID NO: 18)	TCTAACTCCTCA CAAATTCCTAA (SEQ ID NO: 636) GTAGTGTAATAG GGAAAGGGG (SEQ ID NO: 637)		498
(SEQ ID NO: 48)	TAAAATTCCCTC TTACCCTAAA (SEQ ID NO: 638) TAGTAAGGATTG TAGAAGGGGG (SEQ ID NO: 639)		396
(SEQ ID NO:	TAGTAAGGATTG TAGAAGGGGG (SEQ ID NO: 640)		

No:	Gene:	Primer:	Amplificate Length:
48)	CCTCAAACCCTA AAAATAACC (SEQ ID NO: 641)		
(SEQ ID NO: 58)	GGAGAGGAGTG TTTGTAGAAGA (SEQ ID NO: 642) CAATCTCCCCTA AATCCTAAT (SEQ ID NO: 643)		369
(SEQ ID NO: 22)	TAGTAGTTTGAA GAAGGGGAAG (SEQ ID NO: 644) AAACATTCCTAA AATCACAAAAA (SEQ ID NO: 645)		373
(SEQ ID NO: 19)	TTTATTTGGGTA TGATTAGGTTTT (SEQ ID NO: 646) ACTAAAAACACC ACCCCCT (SEQ ID NO: 647)		426
(SEQ ID NO: 19)	ACAAACCAAAAT CTTACTTCCTA (SEQ ID NO: 648) GAATGGAGGGG AAATGTTA (SEQ ID NO: 649)		458
(SEQ ID NO: 17)	AAAACTCCTCCC CTCTATAAAT (SEQ ID NO: 650) TTGGAGAGATGT GTTGGTTAG (SEQ ID NO: 651)		492
(SEQ ID NO: 17)	AATCCTAACCAA CACATCTCTC (SEQ ID NO: 652) AGGGGATTTTAA GGTGATTAGT (SEQ ID NO: 653)		482
(SEQ ID NO: 23)	CTCCCCATCCAT CTTATTTTA (SEQ ID NO: 654) ATTGTTTGGGTG ATAGTGAAGT (SEQ ID NO: 655)		489
(SEQ ID NO: 21)	TAAGTTTTTGGA GGAAGAGTTT (SEQ ID NO: 656) AAAATACTCCCT		370

No:	Gene:	Primer:	Amplificate Length:
	ATAATTCCCC (SEQ ID NO: 657)		
(SEQ ID NO: 21)	TTTCTCTAACCA AACACCTAAAA (SEQ ID NO: 658) AGAAATTAGTAG AGGAGGGAGG (SEQ ID NO: 659)		398
(SEQ ID NO: 43)	ATCTAATCCCTC TCCTAACTCC (SEQ ID NO: 660) TTTGTTTTGGAA TTTAGGTTTT (SEQ ID NO: 661)		441
(SEQ ID NO: 9)	TCCACAAAACTC TCCTACTAAAA (SEQ ID NO: 662) GGAAGGTTGGGT AGATATAGG (SEQ ID NO: 663)		186
(SEQ ID NO: 12)	TTGGTAGAGTTG AAAGGAGATAG (SEQ ID NO: 664) AAAAACATTCCC TAAAAATTCC (SEQ ID NO: 665)		402
(SEQ ID NO: 47)	ATAGAATGGTTA GGGGGTATTT (SEQ ID NO: 666) TACAAATATCAA CCTCTCTCCC (SEQ ID NO: 667)		484
(SEQ ID NO: 20)	GGTGGGGTATAA TAGTAGGGAT (SEQ ID NO: 668) CTTCCCCTCTTTC ATTTTATTT (SEQ ID NO: 669)		448
(SEQ ID NO: 50)	GAGGAATTGGTA TTGAAAGAAA (SEQ ID NO: 670) CTAATCCACCCT CCATAAAAC (SEQ ID NO: 671)		426
(SEQ ID NO: 50)	CTCCAATTCTCC TCCCTATATC (SEQ ID NO: 672) TAATTTTTGAGG TTGGGAAA		425

No:	Gene:	Primer:	Amplificate Length:
	(SEQ ID NO: 673)		

TABLE 2.

No:	Gene	Oligo:
1	(SEQ ID NO: 41)	TGGACGTAGGAAAGCGA (SEQ ID NO: 681)
2	(SEQ ID NO: 41)	GATGTAGGAAAGTGAGA (SEQ ID NO: 682)
3	(SEQ ID NO: 41)	ATTTACGGGAGTTTTATCGT (SEQ ID NO: 683)
4	(SEQ ID NO: 41)	ATTTATGGGAGTTTTATTGT (SEQ ID NO: 684)
5	(SEQ ID NO: 41)	ATTAGTTCGGGTCGCGT (SEQ ID NO: 685)
6	(SEQ ID NO: 41)	ATTAGTTTGGGTTGTGT (SEQ ID NO: 686)
7	(SEQ ID NO: 41)	TATACGAAAGGGAGGCGG (SEQ ID NO: 687)
8	(SEQ ID NO: 41)	TATATGAAAGGGAGGTGG (SEQ ID NO: 688)
9	(SEQ ID NO: 41)	GGCGTGTCGTTAGTTTTA (SEQ ID NO: 689)
10	(SEQ ID NO: 41)	GGTGTGTTGTTAGTTTTATA (SEQ ID NO: 690)
11	(SEQ ID NO: 41)	TTCGATTGACGTTAGCGA (SEQ ID NO: 691)
12	(SEQ ID NO: 41)	TTTGATTGATGTTAGTGA (SEQ ID NO: 692)
13	(SEQ ID NO: 41)	TTTCGAGTTTGACGGT (SEQ ID NO: 693)
14	(SEQ ID NO: 41)	TTTTGAGTTTGATGGTT (SEQ ID NO: 694)
15	(SEQ ID NO: 41)	TTCGGAGGGCGTATTT (SEQ ID NO: 695)
16	(SEQ ID NO: 41)	TTTGGAGGGTGTATTT (SEQ ID NO: 696)
17	(SEQ ID NO: 5)	GACGTCGGTACGTAGT (SEQ ID NO: 697)
18	(SEQ ID NO: 5)	GATGTTGGTATGTAGTAG (SEQ ID NO: 698)
19	(SEQ ID NO: 5)	TTCGGGGGAATTCGAGT (SEQ ID NO: 699)
20	(SEQ ID NO: 5)	TTTGGGGGAATTTGAGT (SEQ ID NO: 700)
21	(SEQ ID NO: 5)	TATTGCGAGGATTCGG (SEQ ID NO: 701)

No:	Gene	Oligo:
22	(SEQ ID NO: 5)	ATTGTGAGGATTTGGT (SEQ ID NO: 702)
23	(SEQ ID NO: 5)	GTGCGTTCGTAGCGTA (SEQ ID NO: 703)
24	(SEQ ID NO: 5)	TGTGTTTGTAGTGTAGG (SEQ ID NO: 704)
25	(SEQ ID NO: 5)	GGACGTCGTTTGTTAG (SEQ ID NO: 705)
26	(SEQ ID NO: 5)	GGATGTTGTTTGTTAGG (SEQ ID NO: 706)
27	(SEQ ID NO: 5)	AGAGCGTCGTTTTGTA (SEQ ID NO: 707)
28	(SEQ ID NO: 5)	AGAGTGTTGTTTTGTAT (SEQ ID NO: 708)
29	(SEQ ID NO: 5)	TTTCGAGGGTAGGCGAG (SEQ ID NO: 709)
30	(SEQ ID NO: 5)	TTTTGAGGGTAGGTGAG (SEQ ID NO: 710)
31	(SEQ ID NO: 5)	TTTCGATTTTAATGCGAA (SEQ ID NO: 711)
32	(SEQ ID NO: 5)	TTTGATTTTAATGTGAAGT (SEQ ID NO: 712)
33	(SEQ ID NO: 5)	AGGAATTTCGTCGCGA (SEQ ID NO: 713)
34	(SEQ ID NO: 5)	AGGAATTTTGTTGTGAT (SEQ ID NO: 714)
35	(SEQ ID NO: 5)	TTTGAGTCGTACGCGT (SEQ ID NO: 715)
36	(SEQ ID NO: 5)	TTTTGAGTTGTATGTGT (SEQ ID NO: 716)
37	(SEQ ID NO: 6)	TACGTAGTTGCGCGTT (SEQ ID NO: 717)
38	(SEQ ID NO: 51)	GTATGTAGTTGTGTGTT (SEQ ID NO: 674)
39	(SEQ ID NO: 6)	AATCGGCGGTTAGGAT (SEQ ID NO: 718)
40	(SEQ ID NO: 6)	GAATTGGTGGTTAGGA (SEQ ID NO: 719)
41	(SEQ ID NO: 6)	TTTGATCGGGTTTGAG (SEQ ID NO: 720)
42	(SEQ ID NO: 6)	TTTTGATTGGGTTTGAG (SEQ ID NO: 721)
43	(SEQ ID NO: 51)	TTTGAGTATTCGTAGGAA (SEQ ID NO: 675)
44	(SEQ ID NO: 6)	TGAGTATTTGTAGGAAGA (SEQ ID NO: 722)
45	(SEQ ID NO: 6)	AGAGGCGCGGGTTATA (SEQ ID NO: 723)
46	(SEQ ID NO: 6)	TAGAGGTGTGGGTTAT (SEQ ID NO: 724)

No:	Gene	Oligo:
47	(SEQ ID NO: 6)	TTAGCGGTTAAGTTGCGA (SEQ ID NO: 725)
48	(SEQ ID NO: 6)	TTAGTGGTTAAGTTGTGA (SEQ ID NO: 726)
49	(SEQ ID NO: 8)	TTCGTAGAAGAATACGCGTA (SEQ ID NO: 727)
50	(SEQ ID NO: 8)	TTTGTAGAAGAATATGTGTA (SEQ ID NO: 728)
51	(SEQ ID NO: 8)	AAACGTTTATCGGTTG (SEQ ID NO: 729)
52	(SEQ ID NO: 8)	AATGTTTATTGGTTGGA (SEQ ID NO: 730)
53	(SEQ ID NO: 8)	TATCGTAGTTCGTTCGG (SEQ ID NO: 731)
54	(SEQ ID NO: 8)	ATTGTAGTTTGTTTGGT (SEQ ID NO: 732)
55	(SEQ ID NO: 16)	TGGTCGGTATATTTCGA (SEQ ID NO: 733)
56	(SEQ ID NO: 16)	TTGGTTGGTATATTTTGA (SEQ ID NO: 734)
57	(SEQ ID NO: 16)	GGAGGTTTCGGTTCGA (SEQ ID NO: 735)
58	(SEQ ID NO: 16)	TGGAGGTTTTGGTTTGA (SEQ ID NO: 736)
59	(SEQ ID NO: 16)	TTAGCGGTAATAGCGG (SEQ ID NO: 737)
60	(SEQ ID NO: 52)	TATTAGTGGTAATAGTGG (SEQ ID NO: 676)
61	(SEQ ID NO: 42)	TGCGTAGTAGGCGGTT (SEQ ID NO: 738)
62	(SEQ ID NO: 53)	TGTGTAGTAGGTGGTTT (SEQ ID NO: 677)
63	(SEQ ID NO: 42)	TAGGCGGTTGTTCGTA (SEQ ID NO: 739)
64	(SEQ ID NO: 42)	AGGTGGTTGTTTGTAA (SEQ ID NO: 740)
65	(SEQ ID NO: 14)	TTGAAGTCGGTACGGT (SEQ ID NO: 1125)
66	(SEQ ID NO: 14)	TGAAGTTGGTATGGTT (SEQ ID NO: 1126)
67	(SEQ ID NO: 14)	TGGGACGCGGATATTT (SEQ ID NO: 1127)
68	(SEQ ID NO: 14)	GTTGGGATGTGGATAT (SEQ ID NO: 1128)
69	(SEQ ID NO: 43)	GTTCGGGTCGATTCGA (SEQ ID NO: 741)
70	(SEQ ID NO: 43)	GGTTTGGGTTGATTTGA (SEQ ID NO: 742)
71	(SEQ ID NO: 43)	TTCGGGATATATTCGATT (SEQ ID NO: 743)

No:	Gene	Oligo:
72	(SEQ ID NO: 43)	TTTTGGGATATATTTGATT (SEQ ID NO: 744)
73	(SEQ ID NO: 43)	TATTCGAATTGTATTCGT (SEQ ID NO: 745)
74	(SEQ ID NO: 43)	TTTGAATTGTATTTGTTTAT (SEQ ID NO: 746)
75	(SEQ ID NO: 15)	TTAAGTTCGATTCGGT (SEQ ID NO: 747)
76	(SEQ ID NO: 54)	AGTTTGATTTGGTGAAT (SEQ ID NO: 678)
77	(SEQ ID NO: 15)	TAGTTGTTCGAGAGGG (SEQ ID NO: 748)
78	(SEQ ID NO: 15)	AGTTGTTTGAGAGGGT (SEQ ID NO: 749)
79	(SEQ ID NO: 15)	ATAGTATCGAGGTGAGT (SEQ ID NO: 750)
80	(SEQ ID NO: 15)	ATAGTATTGAGGTGAGTT (SEQ ID NO: 751)
81	(SEQ ID NO: 15)	TTCGGGATTCGATAAT (SEQ ID NO: 752)
82	(SEQ ID NO: 15)	AGGTTTTGGGATTTGA (SEQ ID NO: 753)
83	(SEQ ID NO: 15)	TTAGGACGCGGCGATA (SEQ ID NO: 754)
84	(SEQ ID NO: 15)	AGGATGTGGTGATAGT (SEQ ID NO: 755)
85	(SEQ ID NO: 15)	TTGTACGTTCGGTATT (SEQ ID NO: 756)
86	(SEQ ID NO: 15)	TAAGTTGTATGTTTGGTA (SEQ ID NO: 757)
87	(SEQ ID NO: 4)	GGCGTCGAGGTCGTAG (SEQ ID NO: 758)
88	(SEQ ID NO: 4)	GGTGTTGAGGTTGTAGT (SEQ ID NO: 759)
89	(SEQ ID NO: 4)	AGGGTTTCGATTTTCGG (SEQ ID NO: 760)
90	(SEQ ID NO: 4)	AGGGTTTTGATTTTTGG (SEQ ID NO: 761)
91	(SEQ ID NO: 4)	TGGAACGTGCGATTGT (SEQ ID NO: 762)
92	(SEQ ID NO: 4)	TGGAATGTGTGATTGT (SEQ ID NO: 763)
93	(SEQ ID NO: 4)	TTTTGGCGCGTTTATA (SEQ ID NO: 764)
94	(SEQ ID NO: 4)	TTGGTGTGTTTATAGATA (SEQ ID NO: 765)
95	(SEQ ID NO: 4)	AGATTTTTACGATTCGA (SEQ ID NO: 766)
96	(SEQ ID NO: 4)	TTTTTATGATTTGAAATAGA (SEQ ID NO: 767)

No:	Gene	Oligo:
97	(SEQ ID NO: 4)	AGTATTTTCGCGTGTT (SEQ ID NO: 768)
98	(SEQ ID NO: 4)	TAGTATTTTTGTGTGTTAA (SEQ ID NO: 769)
99	(SEQ ID NO: 7)	TTCGTCGGCGGTAGAG (SEQ ID NO: 770)
100	(SEQ ID NO: 7)	TAGAGTTTGTTGGTGG (SEQ ID NO: 771)
101	(SEQ ID NO: 7)	GATCGCGGGTACGTTT (SEQ ID NO: 772)
102	(SEQ ID NO: 7)	ATTGTGGGTATGTTTGT (SEQ ID NO: 773)
103	(SEQ ID NO: 7)	TTAACGTCGTTGGTTA (SEQ ID NO: 774)
104	(SEQ ID NO: 7)	TGATTAATGTTGTTGGT (SEQ ID NO: 775)
105	(SEQ ID NO: 7)	TTCGCGCGAAGATTTA (SEQ ID NO: 776)
106	(SEQ ID NO: 7)	GTTTTTGTGTGAAGATT (SEQ ID NO: 777)
107	(SEQ ID NO: 44)	TTCGATATCGTGACGG (SEQ ID NO: 778)
108	(SEQ ID NO: 44)	TTTTGATATTGTGATGGT (SEQ ID NO: 779)
109	(SEQ ID NO: 44)	AGAATACGGTCGTAGA (SEQ ID NO: 780)
110	(SEQ ID NO: 44)	TAGAATATGGTTGTAGATA (SEQ ID NO: 781)
111	(SEQ ID NO: 44)	TATTTTTGCGTACGGG (SEQ ID NO: 782)
112	(SEQ ID NO: 44)	ATTTTTGTGTATGGGTT (SEQ ID NO: 783)
113	(SEQ ID NO: 1)	TTACGGTGAAGGCGGA (SEQ ID NO: 784)
114	(SEQ ID NO: 1)	TTATGGTGAAGGTGGA (SEQ ID NO: 785)
115	(SEQ ID NO: 1)	TTCGGGATTAATATCGAGAT (SEQ ID NO: 786)
116	(SEQ ID NO: 1)	TTTGGGATTAATATTGAGAT (SEQ ID NO: 787)
117	(SEQ ID NO: 1)	TTTCGGTTTTCGTTAAT (SEQ ID NO: 788)
118	(SEQ ID NO: 1)	TTTGGTTTTTGTTAATTTAG (SEQ ID NO: 789)
119	(SEQ ID NO: 1)	TGTGCGAAGTTAACGT (SEQ ID NO: 790)
120	(SEQ ID NO: 1)	TTGTGTGAAGTTAATGT (SEQ ID NO: 791)
121	(SEQ ID NO: 1)	AAGTTTATCGGCGTTT (SEQ ID NO: 792)

No:	Gene	Oligo:
122	(SEQ ID NO: 1)	AGAAGTTTATTGGTGTTT (SEQ ID NO: 793)
123	(SEQ ID NO: 1)	ATTTCGGAATTTAAGCGT (SEQ ID NO: 794)
124	(SEQ ID NO: 1)	TTTGGAATTTAAGTGTTT (SEQ ID NO: 795)
125	(SEQ ID NO: 1)	TTTTCGCGATTGGAGA (SEQ ID NO: 796)
126	(SEQ ID NO: 1)	GTTTTTGTGATTGGAGA (SEQ ID NO: 797)
127	(SEQ ID NO: 1)	ATTTACGCGTTTTAGG (SEQ ID NO: 798)
128	(SEQ ID NO: 1)	ATGGAATTTATGTGTTTT (SEQ ID NO: 799)
129	(SEQ ID NO: 1)	ATGTCGCGGTTTTATA (SEQ ID NO: 800)
130	(SEQ ID NO: 1)	GGATGTTGTGGTTTTAT (SEQ ID NO: 801)
131	(SEQ ID NO: 2)	AGACGGGGTTTACGAG (SEQ ID NO: 802)
132	(SEQ ID NO: 2)	AGATGGGGTTTATGAG (SEQ ID NO: 803)
133	(SEQ ID NO: 2)	TGTCGGTATTAGCGTT (SEQ ID NO: 804)
134	(SEQ ID NO: 2)	GTGTTGGTATTAGTGTT (SEQ ID NO: 805)
135	(SEQ ID NO: 2)	TGGTTTACGTTCGGTA (SEQ ID NO: 806)
136	(SEQ ID NO: 2)	GGTTTATGTTTGGTAGT (SEQ ID NO: 807)
137	(SEQ ID NO: 2)	TTCGTACGGTTAGGTT (SEQ ID NO: 808)
138	(SEQ ID NO: 2)	AGTTTTGTATGGTTAGG (SEQ ID NO: 809)
139	(SEQ ID NO: 45)	ATAGCGATTTCGGCGA (SEQ ID NO: 810)
140	(SEQ ID NO: 45)	AGTGATTTTGGTGAGA (SEQ ID NO: 811)
141	(SEQ ID NO: 45)	GGCGTTTTATTTACGAGA (SEQ ID NO: 812)
142	(SEQ ID NO: 45)	GGGTGTTTTATTTATGAG (SEQ ID NO: 813)
143	(SEQ ID NO: 45)	ATCGTGGACGGTAACGA (SEQ ID NO: 814)
144	(SEQ ID NO: 45)	ATTGTGGATGGTAATGA (SEQ ID NO: 815)
145	(SEQ ID NO: 45)	TTGAGATCGATTCGTT (SEQ ID NO: 816)
146	(SEQ ID NO: 45)	TGAGATTGATTTGTTTAG (SEQ ID NO: 817)

No:	Gene	Oligo:
147	(SEQ ID NO: 45)	GGCGAGATTCGTACGT (SEQ ID NO: 818)
148	(SEQ ID NO: 45)	GGTGAGATTTGTATGTT (SEQ ID NO: 819)
149	(SEQ ID NO: 45)	TGACGTTCGTGGTGGA (SEQ ID NO: 820)
150	(SEQ ID NO: 45)	GATGTTTGTGGTGGAG (SEQ ID NO: 821)
151	(SEQ ID NO: 45)	GTGATCGATTACGGTA (SEQ ID NO: 822)
152	(SEQ ID NO: 45)	AGGTGATTGATTATGGT (SEQ ID NO: 823)
153	(SEQ ID NO: 45)	ATTATTCGTTCGGTGA (SEQ ID NO: 824)
154	(SEQ ID NO: 45)	TATTATTTGTTTGGTGAG (SEQ ID NO: 825)
155	(SEQ ID NO: 45)	TATCGTCGTTAAGTGT (SEQ ID NO: 826)
156	(SEQ ID NO: 45)	TATTATTGTTGTTAAGTGT (SEQ ID NO: 827)
157	(SEQ ID NO: 45)	TGTAAGCGCGAGAATA (SEQ ID NO: 828)
158	(SEQ ID NO: 45)	AGTGTAAGTGTGAGAAT (SEQ ID NO: 829)
159	(SEQ ID NO: 9)	TATAGCGGTTTACGGT (SEQ ID NO: 830)
160	(SEQ ID NO: 9)	TAGTGGTTTATGGTAGT (SEQ ID NO: 831)
161	(SEQ ID NO: 9)	AGGGCGATTAGGACGT (SEQ ID NO: 832)
162	(SEQ ID NO: 9)	AGGGTGATTAGGATGT (SEQ ID NO: 833)
163	(SEQ ID NO: 46)	TTCGTTAGAGTTCGTAG (SEQ ID NO: 834)
164	(SEQ ID NO: 46)	TTTGTTAGAGTTTGTAGT (SEQ ID NO: 835)
165	(SEQ ID NO: 46)	TGAGACGTTTGTCGGT (SEQ ID NO: 836)
166	(SEQ ID NO: 46)	TGAGATGTTTGTTGGT (SEQ ID NO: 837)
167	(SEQ ID NO: 46)	GAAAAGTTCGTCGGTT (SEQ ID NO: 838)
168	(SEQ ID NO: 46)	AGAAAAGTTTGTTGGTT (SEQ ID NO: 839)
169	(SEQ ID NO: 46)	ATGGCGTAGTCGCGAT (SEQ ID NO: 840)
170	(SEQ ID NO: 46)	TGGTGTAGTTGTGATT (SEQ ID NO: 841)
171	(SEQ ID NO: 10)	TTTTGACGTCGATGTA (SEQ ID NO: 842)

No:	Gene	Oligo:
172	(SEQ ID NO: 10)	TGATGTTGATGTAGAATT (SEQ ID NO: 843)
173	(SEQ ID NO: 10)	TTGCGATGTGCGTTTA (SEQ ID NO: 844)
174	(SEQ ID NO: 10)	TGTGATGTGTGTTTAGT (SEQ ID NO: 845)
175	(SEQ ID NO: 10)	TGATTACGGCGCGGAT (SEQ ID NO: 846)
176	(SEQ ID NO: 10)	ATTATGGTGTGGATGG (SEQ ID NO: 847)
177	(SEQ ID NO: 10)	AGATGGCGACGTCGAA (SEQ ID NO: 848)
178	(SEQ ID NO: 10)	ATGGTGATGTTGAAGA (SEQ ID NO: 849)
179	(SEQ ID NO: 10)	TTTAAGCGCGGCGGTA (SEQ ID NO: 850)
180	(SEQ ID NO: 10)	TTTTAAGTGTGGTGGTA (SEQ ID NO: 851)
181	(SEQ ID NO: 10)	AGAAACGTAGACGCGA (SEQ ID NO: 852)
182	(SEQ ID NO: 10)	AATGTAGATGTGATGGA (SEQ ID NO: 853)
183	(SEQ ID NO: 11)	AGAGACGCGAAAAATT (SEQ ID NO: 854)
184	(SEQ ID NO: 11)	TAGAGAGATGTGAAAAAT (SEQ ID NO: 855)
185	(SEQ ID NO: 11)	AGACGAAAGAGTCGTT (SEQ ID NO: 856)
186	(SEQ ID NO: 11)	AGAGATGAAAGAGTTGT (SEQ ID NO: 857)
187	(SEQ ID NO: 11)	TTTTAGTTCGAGCGTA (SEQ ID NO: 858)
188	(SEQ ID NO: 11)	TTAGTTTGAGTGTAGTTA (SEQ ID NO: 859)
189	(SEQ ID NO: 11)	GACGTGAATTTTCGGAA (SEQ ID NO: 860)
190	(SEQ ID NO: 11)	AGGATGTGAATTTTTGG (SEQ ID NO: 861)
191	(SEQ ID NO: 11)	AATGCGTGGTCGTTTT (SEQ ID NO: 862)
192	(SEQ ID NO: 11)	GAAATGTGTGGTTGTT (SEQ ID NO: 863)
193	(SEQ ID NO: 11)	TTTCGTTTGCGGAATT (SEQ ID NO: 864)
194	(SEQ ID NO: 11)	TTTTGTTTGTGGAATTG (SEQ ID NO: 865)
195	(SEQ ID NO: 12)	TGTTCGACGTGATTTT (SEQ ID NO: 866)
196	(SEQ ID NO: 12)	GTGTTTGATGTGATTTT (SEQ ID NO: 867)

No:	Gene	Oligo:
197	(SEQ ID NO: 12)	TAACGTTTTTTCGGGT (SEQ ID NO: 868)
198	(SEQ ID NO: 12)	TTAATGTTTTTTGGGTG (SEQ ID NO: 869)
199	(SEQ ID NO: 12)	TGTTGATTCGGAAATGA (SEQ ID NO: 870)
200	(SEQ ID NO: 12)	TTTGTTGATTTGGAAATG (SEQ ID NO: 871)
201	(SEQ ID NO: 13)	TAAAGTTTCGAAGCGG (SEQ ID NO: 1129)
202	(SEQ ID NO: 13)	AGTTTTGAAGTGGAGT (SEQ ID NO: 1130)
203	(SEQ ID NO: 13)	AAGTCGGTAGTTATCGT (SEQ ID NO: 1131)
204	(SEQ ID NO: 13)	AAGTTGGTAGTTATTGTT (SEQ ID NO: 1132)
205	(SEQ ID NO: 3)	TTGGAGCGCGAGAAAG (SEQ ID NO: 872)
206	(SEQ ID NO: 3)	TTGGAGTGTGAGAAAG (SEQ ID NO: 873)
207	(SEQ ID NO: 3)	TACGTTATCGGTTCGT (SEQ ID NO: 874)
208	(SEQ ID NO: 3)	TATGTTATTGGTTTGTATT (SEQ ID NO: 875)
209	(SEQ ID NO: 3)	ATTAGGTTCGTGGGCGT (SEQ ID NO: 876)
210	(SEQ ID NO: 3)	ATTAGGTTTGTGGGTGT (SEQ ID NO: 877)
211	(SEQ ID NO: 3)	TGCGGTTTAGAAACGTAG (SEQ ID NO: 878)
212	(SEQ ID NO: 3)	TGTGGTTTAGAAATGTAG (SEQ ID NO: 879)
213	(SEQ ID NO: 3)	GAACGGGTTTCGTAGT (SEQ ID NO: 880)
214	(SEQ ID NO: 3)	GGGAATGGGTTTTGTA (SEQ ID NO: 881)
215	(SEQ ID NO: 3)	TTGCGATAGTCGGCGG (SEQ ID NO: 882)
216	(SEQ ID NO: 3)	TTGTGATAGTTGGTGG (SEQ ID NO: 883)
217	(SEQ ID NO: 3)	AAGAACGGACGTGTTT (SEQ ID NO: 884)
218	(SEQ ID NO: 3)	AGGAAGAATGGATGTG (SEQ ID NO: 885)
219	(SEQ ID NO: 47)	AAGTTTCGTTTGGGAG (SEQ ID NO: 886)
220	(SEQ ID NO: 47)	AAAGTTTTGTTTGGGAG (SEQ ID NO: 887)
221	(SEQ ID NO: 47)	TTGGAAGTCGAAGAGA (SEQ ID NO: 888)

No:	Gene	Oligo:
222	(SEQ ID NO: 47)	TTTGGAAGTTGAAGAGA (SEQ ID NO: 889)
223	(SEQ ID NO: 18)	TATCGGGTTCGATTTT (SEQ ID NO: 890)
224	(SEQ ID NO: 18)	GGTGTATTGGGTTTGA (SEQ ID NO: 891)
225	(SEQ ID NO: 20)	TAGGGATTCGCGGAGG (SEQ ID NO: 892)
226	(SEQ ID NO: 20)	TAGGGATTTGTGGAGG (SEQ ID NO: 893)
227	(SEQ ID NO: 20)	TTGTCGAGTAATTTTCGT (SEQ ID NO: 894)
228	(SEQ ID NO: 20)	TGTTGAGTAATTTTTGTT (SEQ ID NO: 895)
229	(SEQ ID NO: 20)	TATTACGGGCGGAGGG (SEQ ID NO: 896)
230	(SEQ ID NO: 20)	TATTATGGGTGGAGGG (SEQ ID NO: 897)
231	(SEQ ID NO: 20)	GACGGTACGTTAGAGG (SEQ ID NO: 898)
232	(SEQ ID NO: 20)	GATGGTATGTTAGAGGT (SEQ ID NO: 899)
233	(SEQ ID NO: 20)	TTGGGCGTCGTTATTA (SEQ ID NO: 900)
234	(SEQ ID NO: 20)	TGGGTGTTGTTATTAGT (SEQ ID NO: 901)
235	(SEQ ID NO: 20)	TATTAGTTCGGTCGTT (SEQ ID NO: 902)
236	(SEQ ID NO: 20)	AGTTTGGTTGTTAGTTT (SEQ ID NO: 903)
237	(SEQ ID NO: 20)	TTATTACGTTTAGCGAT (SEQ ID NO: 904)
238	(SEQ ID NO: 20)	TTTTTATTATGTTTAGTGATA (SEQ ID NO: 905)
239	(SEQ ID NO: 20)	ATAGCGAGTGCGATAT (SEQ ID NO: 906)
240	(SEQ ID NO: 48)	AGGGTCGTAGCGGTAG (SEQ ID NO: 907)
241	(SEQ ID NO: 48)	GAGGGTTGTAGTGGTA (SEQ ID NO: 908)
242	(SEQ ID NO: 50)	TTAGGTCGGACGTAAG (SEQ ID NO: 1143)
243	(SEQ ID NO: 50)	GGTTAGGTTGGATGTA (SEQ ID NO: 1144)
244	(SEQ ID NO: 22)	TAGACGTGGGGTTACGT (SEQ ID NO: 909)
245	(SEQ ID NO: 22)	TAGATGTGGGGTTATGT (SEQ ID NO: 910)
246	(SEQ ID NO: 22)	ATTTCGGGGTAGTATCGT (SEQ ID NO: 911)

No:	Gene	Oligo:
247	(SEQ ID NO: 22)	ATTTTGGGGTAGTATTGT (SEQ ID NO: 912)
248	(SEQ ID NO: 19)	TACGCGCGTTTTAAAA (SEQ ID NO: 913)
249	(SEQ ID NO: 19)	TTATGTGTGTTTTAAAATG (SEQ ID NO: 914)
250	(SEQ ID NO: 19)	TACGATATCGTTATATAACGG (SEQ ID NO: 915)
251	(SEQ ID NO: 19)	TATGATATTGTTATATAATGG (SEQ ID NO: 916)
252	(SEQ ID NO: 19)	TATAGGTTCGCGGTTT (SEQ ID NO: 917)
253	(SEQ ID NO: 19)	TATATAGGTTTGTGGTTT (SEQ ID NO: 918)
254	(SEQ ID NO: 19)	TAGGTGCGCGTTATAT (SEQ ID NO: 919)
255	(SEQ ID NO: 19)	ATGTAGGTGTGTGTTAT (SEQ ID NO: 920)
256	(SEQ ID NO: 55)	TACGTTGTTTGGACGAAT (SEQ ID NO: 679)
257	(SEQ ID NO: 19)	TATGTTGTTTGGATGAAT (SEQ ID NO: 921)
258	(SEQ ID NO: 19)	AAGGAGCGTATTTCGG (SEQ ID NO: 922)
259	(SEQ ID NO: 19)	AGGAGTGTATTTTGGG (SEQ ID NO: 923)
260	(SEQ ID NO: 55)	GTCGGATTTCGGAAGT (SEQ ID NO: 680)
261	(SEQ ID NO: 19)	GTTGGATTTTGGAAGTG (SEQ ID NO: 924)
262	(SEQ ID NO: 19)	GAAGTGACGCGTTCGT (SEQ ID NO: 925)
263	(SEQ ID NO: 19)	GAAGTGATGTGTTTGT (SEQ ID NO: 926)
264	(SEQ ID NO: 19)	TGTTATCGTTGCGCGA (SEQ ID NO: 927)
265	(SEQ ID NO: 19)	ATGTTATTGTTGTGTGA (SEQ ID NO: 928)
266	(SEQ ID NO: 17)	TGAAAACGTTTTTCGT (SEQ ID NO: 929)
267	(SEQ ID NO: 17)	AATGTTTTTTGTAAAGAAA (SEQ ID NO: 930)
268	(SEQ ID NO: 17)	AGGATTTCGGCGTTAT (SEQ ID NO: 931)
269	(SEQ ID NO: 17)	AAAGGATTTTGGTGTTA (SEQ ID NO: 932)
270	(SEQ ID NO: 17)	ATTTATTCGTGCGTTT (SEQ ID NO: 933)
271	(SEQ ID NO: 17)	TATTTGTGTGTTTAGGG (SEQ ID NO: 934)

No:	Gene	Oligo:
272	(SEQ ID NO: 17)	TTTCGGTGGTTTTCGAA (SEQ ID NO: 935)
273	(SEQ ID NO: 17)	TTTGGTGGTTTTTGAAG (SEQ ID NO: 936)
274	(SEQ ID NO: 17)	GGCGTACGGAATTTTA (SEQ ID NO: 937)
275	(SEQ ID NO: 17)	GGGTGTATGGAATTTTA (SEQ ID NO: 938)
276	(SEQ ID NO: 17)	TGGACGGAGGTTTCGT (SEQ ID NO: 939)
277	(SEQ ID NO: 17)	TGGATGGAGGTTTTGT (SEQ ID NO: 940)
278	(SEQ ID NO: 17)	TGCGGACGGGATAGTT (SEQ ID NO: 941)
279	(SEQ ID NO: 17)	TGTGGATGGGATAGTT (SEQ ID NO: 942)
280	(SEQ ID NO: 17)	TGATTAGTCGATTCGT (SEQ ID NO: 943)
281	(SEQ ID NO: 17)	GATGTAGGGATGGAGA (SEQ ID NO: 944)
282	(SEQ ID NO: 17)	TATCGTGGTTTTTTACGTAT (SEQ ID NO: 945)
283	(SEQ ID NO: 17)	ATATTGTGGTTTTTTATGTA (SEQ ID NO: 946)
284	(SEQ ID NO: 17)	TTTATTCGGTGTTCGA (SEQ ID NO: 947)
285	(SEQ ID NO: 17)	TATTTGGTGTTTGAGAG (SEQ ID NO: 948)
286	(SEQ ID NO: 23)	GAGGCGCGTTATTTTT (SEQ ID NO: 1133)
287	(SEQ ID NO: 23)	GGGAGGTGTGTTATTTT (SEQ ID NO: 1134)
288	(SEQ ID NO: 23)	AACGGTAGTTAGCGATA (SEQ ID NO: 1135)
289	(SEQ ID NO: 23)	TGAATGGTAGTTAGTGA (SEQ ID NO: 1136)
290	(SEQ ID NO: 23)	TTTTAACGTTCGCGGA (SEQ ID NO: 1137)
291	(SEQ ID NO: 23)	AATGTTTGTGGAGGAT (SEQ ID NO: 1138)
292	(SEQ ID NO: 21)	TTTTTCGCGTATATGTT (SEQ ID NO: 1139)
293	(SEQ ID NO: 21)	TTTTTTGTGTATATGTTTAGG (SEQ ID NO: 1140)
294	(SEQ ID NO: 21)	AAGGGCGGTAAGACGG (SEQ ID NO: 1141)
295	(SEQ ID NO: 21)	AAGGGTGGTAAGATGG (SEQ ID NO: 1142)
296	(SEQ ID NO: 32)	GGTTTCGTTTAATCGT (SEQ ID NO: 949)

No:	Gene	Oligo:
297	(SEQ ID NO: 32)	GGGTTTTGTTTAATTGTA (SEQ ID NO: 950)
298	(SEQ ID NO: 32)	GATTCGTATTTCGTAGT (SEQ ID NO: 951)
299	(SEQ ID NO: 32)	TTTGTATTTTGTAGTGGG (SEQ ID NO: 952)
300	(SEQ ID NO: 32)	TTCGTATTTAGCGGAT (SEQ ID NO: 953)
301	(SEQ ID NO: 32)	GGTTTGTATTTAGTGGA (SEQ ID NO: 954)
302	(SEQ ID NO: 32)	TTAATCGGCGGGTTTT (SEQ ID NO: 955)
303	(SEQ ID NO: 32)	AGTTAATTGGTGGGTT (SEQ ID NO: 956)
304	(SEQ ID NO: 32)	TATTTTGGCGGGTTGTAT (SEQ ID NO: 957)
305	(SEQ ID NO: 32)	TATTTTGGTGGGTTGTAT (SEQ ID NO: 958)
306	(SEQ ID NO: 32)	AAGGTTATCGGTTTAAGA (SEQ ID NO: 959)
307	(SEQ ID NO: 32)	AAGGTTATTGGTTTAAGA (SEQ ID NO: 960)
308	(SEQ ID NO: 32)	GGGGGACGACGTTTTTGT (SEQ ID NO: 961)
309	(SEQ ID NO: 32)	GGGGGATGATGTTTTTGT (SEQ ID NO: 962)
310	(SEQ ID NO: 33)	TTACGGTTCGGTTATT (SEQ ID NO: 963)
311	(SEQ ID NO: 33)	AGGTTTTATGGTTTGGT (SEQ ID NO: 964)
312	(SEQ ID NO: 33)	GACGTCGCGGGGTTAG (SEQ ID NO: 965)
313	(SEQ ID NO: 33)	TGATGTTGTGGGGTTA (SEQ ID NO: 966)
314	(SEQ ID NO: 33)	AGGTATTTCGCGATAT (SEQ ID NO: 967)
315	(SEQ ID NO: 33)	AGGTATTTTGTGATATTTT (SEQ ID NO: 968)
316	(SEQ ID NO: 33)	GTTTTTCGATTTACGTT (SEQ ID NO: 969)
317	(SEQ ID NO: 33)	TAGGTTTTTTGATTTATGT (SEQ ID NO: 970)
318	(SEQ ID NO: 33)	GGTAGTTTCGATTATTTA (SEQ ID NO: 971)
319	(SEQ ID NO: 33)	GGTAGTTTTGATTATTTA (SEQ ID NO: 972)
320	(SEQ ID NO: 33)	TAGAGTACGGGGCGGG (SEQ ID NO: 973)
321	(SEQ ID NO: 33)	TAGAGTATGGGGTGGG (SEQ ID NO: 974)

No:	Gene	Oligo:
322	(SEQ ID NO: 33)	TTGTTTAGCGGATTTTAG (SEQ ID NO: 975)
323	(SEQ ID NO: 33)	TTGTTTAGTGGATTTTAG (SEQ ID NO: 976)
324	(SEQ ID NO: 33)	TAGGTTCGGTTCGTTAT (SEQ ID NO: 977)
325	(SEQ ID NO: 33)	TAGGTTTGGTTTGTTATT (SEQ ID NO: 978)
326	(SEQ ID NO: 33)	TGGTGGTACGTAGTTTGG (SEQ ID NO: 979)
327	(SEQ ID NO: 33)	TTTGGCGTAGATCGGT (SEQ ID NO: 980)
328	(SEQ ID NO: 33)	TTTGGTGTAGATTGGT (SEQ ID NO: 981)
329	(SEQ ID NO: 33)	AGTGTTCGTCGTAGTT (SEQ ID NO: 982)
330	(SEQ ID NO: 33)	TGAGTGTTTGTTGTAGT (SEQ ID NO: 983)
331	(SEQ ID NO: 33)	GTGTTTAGCGCGGATT (SEQ ID NO: 984)
332	(SEQ ID NO: 33)	GGTGTTTAGTGTGGATT (SEQ ID NO: 985)
333	(SEQ ID NO: 34)	TTCGGTTAGTTTCGTAT (SEQ ID NO: 986)
334	(SEQ ID NO: 34)	TTTTGGTTAGTTTTGTATT (SEQ ID NO: 987)
335	(SEQ ID NO: 34)	GATTCGTTTGGGTAACGT (SEQ ID NO: 988)
336	(SEQ ID NO: 34)	GATTTGTTTGGGTAATGT (SEQ ID NO: 989)
337	(SEQ ID NO: 34)	GTCGAATTTAGTCGCGT (SEQ ID NO: 990)
338	(SEQ ID NO: 34)	GTTGAATTTAGTTGTGTA (SEQ ID NO: 991)
339	(SEQ ID NO: 34)	AATTCGCGAGTTTAGA (SEQ ID NO: 992)
340	(SEQ ID NO: 34)	AAAAATTTGTGAGTTTAG (SEQ ID NO: 993)
341	(SEQ ID NO: 24)	AGGGGTTCGATTAGGG (SEQ ID NO: 1145)
342	(SEQ ID NO: 24)	AGGGGTTTGATTAGGG (SEQ ID NO: 1146)
343	(SEQ ID NO: 24)	TTAGGTATACGAAAGAGTAT (SEQ ID NO: 1147)
344	(SEQ ID NO: 24)	TTAGGTATATGAAAGAGTAT (SEQ ID NO: 1148)
345	(SEQ ID NO: 24)	TGTCGTACGTTATGTT (SEQ ID NO: 1149)
346	(SEQ ID NO: 24)	GGTGTTGTATGTTATGT (SEQ ID NO: 1150)

No:	Gene	Oligo:
347	(SEQ ID NO: 24)	TTGATTGGCGGACGAG (SEQ ID NO: 1151)
348	(SEQ ID NO: 24)	TTGATTGGTGGATGAG (SEQ ID NO: 1152)
349	(SEQ ID NO: 35)	TATATATACGTGTGGGTA (SEQ ID NO: 994)
350	(SEQ ID NO: 35)	TATATATATGTGTGGGTA (SEQ ID NO: 995)
351	(SEQ ID NO: 35)	TATGTAGTCGCGTAGT (SEQ ID NO: 996)
352	(SEQ ID NO: 35)	TTTATGTAGTTGTGTAGT (SEQ ID NO: 997)
353	(SEQ ID NO: 35)	AGTGTATGCGTAGAAGGT (SEQ ID NO: 998)
354	(SEQ ID NO: 35)	AGTGTATGTGTAGAAGGT (SEQ ID NO: 999)
355	(SEQ ID NO: 35)	TTTAGATACGAAATGTTA (SEQ ID NO: 1000)
356	(SEQ ID NO: 35)	TTTAGATATGAAATGTTA (SEQ ID NO: 1001)
357	(SEQ ID NO: 35)	AAGTAAGTCGTTGTTGTT (SEQ ID NO: 1002)
358	(SEQ ID NO: 35)	AAGTAAGTTGTTGTTGTT (SEQ ID NO: 1003)
359	(SEQ ID NO: 25)	TTTCGTCGGAGGAATT (SEQ ID NO: 1004)
360	(SEQ ID NO: 25)	GTTTTGTTGGAGGAATT (SEQ ID NO: 1005)
361	(SEQ ID NO: 25)	ATCGTTTTGTCGGACGG (SEQ ID NO: 1006)
362	(SEQ ID NO: 25)	ATTGTTTTGTTGGATGG (SEQ ID NO: 1007)
363	(SEQ ID NO: 25)	TGTCGCGATATATCGA (SEQ ID NO: 1008)
364	(SEQ ID NO: 25)	TTTGTTGTGATATATTGAT (SEQ ID NO: 1009)
365	(SEQ ID NO: 36)	AGCGTCGATTAATCGT (SEQ ID NO: 1010)
366	(SEQ ID NO: 36)	TTAAGTGTTGATTAATTGT (SEQ ID NO: 1011)
367	(SEQ ID NO: 36)	TTCGGTCGGGTTTAAA (SEQ ID NO: 1012)
368	(SEQ ID NO: 36)	GTTTGGTTGGGTTTAAA (SEQ ID NO: 1013)
369	(SEQ ID NO: 36)	TAATCGTTAGCGGCGG (SEQ ID NO: 1014)
370	(SEQ ID NO: 36)	TTAATTGTTAGTGGTGG (SEQ ID NO: 1015)
371	(SEQ ID NO: 36)	TTAACGGGTGGGTACGT (SEQ ID NO: 1016)

No:	Gene	Oligo:
372	(SEQ ID NO: 36)	TTAATGGGTGGGTATGT (SEQ ID NO: 1017)
373	(SEQ ID NO: 36)	AGGTCGTTGGTATTCGT (SEQ ID NO: 1018)
374	(SEQ ID NO: 36)	AGGTTGTTGGTATTTGT (SEQ ID NO: 1019)
375	(SEQ ID NO: 36)	TTTTCGAGTTTTATCGA (SEQ ID NO: 1020)
376	(SEQ ID NO: 36)	TTTGAGTTTTATTGAGGT (SEQ ID NO: 1021)
377	(SEQ ID NO: 36)	ATAGTCGTGGTTTCGT (SEQ ID NO: 1022)
378	(SEQ ID NO: 36)	ATAGTTGTGGTTTTGTT (SEQ ID NO: 1023)
379	(SEQ ID NO: 36)	TGACGGGCGTTTTCGA (SEQ ID NO: 1024)
380	(SEQ ID NO: 36)	GATGGGTGTTTTTGAG (SEQ ID NO: 1025)
381	(SEQ ID NO: 36)	TAATGAGCGCGTTGTA (SEQ ID NO: 1026)
382	(SEQ ID NO: 36)	ATGAGTGTGTTGTATTT (SEQ ID NO: 1027)
383	(SEQ ID NO: 28)	TTGGTTCGGGAAAGGTAA (SEQ ID NO: 1028)
384	(SEQ ID NO: 28)	TTGGTTTGGGAAAGGTAA (SEQ ID NO: 1029)
385	(SEQ ID NO: 28)	TTTCGGTGAATCGGAT (SEQ ID NO: 1030)
386	(SEQ ID NO: 28)	TTTTTGGTGAATTGGAT (SEQ ID NO: 1031)
387	(SEQ ID NO: 28)	TTCGTAAAGTCGTTGT (SEQ ID NO: 1032)
388	(SEQ ID NO: 28)	GGTTTTTTGTAAAGTTGT (SEQ ID NO: 1033)
389	(SEQ ID NO: 28)	GTTTAGTTAGCGGGTTTT (SEQ ID NO: 1034)
390	(SEQ ID NO: 28)	GTTTAGTTAGTGGGTTTT (SEQ ID NO: 1035)
391	(SEQ ID NO: 28)	GGGCGCGTACGGTTAT (SEQ ID NO: 1036)
392	(SEQ ID NO: 28)	AGTTGGGTGTGTATGG (SEQ ID NO: 1037)
393	(SEQ ID NO: 28)	TTATCGCGCGTGGAGG (SEQ ID NO: 1038)
394	(SEQ ID NO: 28)	TTATTGTGTGTGGAGGA (SEQ ID NO: 1039)
395	(SEQ ID NO: 37)	AAAACGTGGACGTTTT (SEQ ID NO: 1153)
396	(SEQ ID NO: 37)	ATTTGGAGCGAGGAATTT (SEQ ID NO: 1154)

No:	Gene	Oligo:
397	(SEQ ID NO: 37)	ATTTGGAGTGAGGAATTT (SEQ ID NO: 1155)
398	(SEQ ID NO: 37)	TTGAAAGTCGGTTAAAGT (SEQ ID NO: 1156)
399	(SEQ ID NO: 37)	TTGAAAGTTGGTTAAAGT (SEQ ID NO: 1157)
400	(SEQ ID NO: 37)	GGTAGTTACGAGAGAGTT (SEQ ID NO: 1158)
401	(SEQ ID NO: 37)	GGTAGTTATGAGAGAGTT (SEQ ID NO: 1159)
402	(SEQ ID NO: 26)	GGTGCGCGTAGAGAAT (SEQ ID NO: 1040)
403	(SEQ ID NO: 26)	GGTGTGTGTAGAGAATA (SEQ ID NO: 1041)
404	(SEQ ID NO: 26)	TAAGCGTATCGACGTT (SEQ ID NO: 1042)
405	(SEQ ID NO: 26)	ATTTTAAGTGTATTGATGT (SEQ ID NO: 1043)
406	(SEQ ID NO: 26)	AAATATCGAACGGGAT (SEQ ID NO: 1044)
407	(SEQ ID NO: 26)	ATTGAATGGGATTTAGAG (SEQ ID NO: 1045)
408	(SEQ ID NO: 26)	TTAGAGTTCGAGTTTATA (SEQ ID NO: 1046)
409	(SEQ ID NO: 26)	TTAGAGTTTGAGTTTATA (SEQ ID NO: 1047)
410	(SEQ ID NO: 26)	TTAGGCGCGGATTCGT (SEQ ID NO: 1048)
411	(SEQ ID NO: 26)	TAGGTGTGGATTTGTT (SEQ ID NO: 1049)
412	(SEQ ID NO: 26)	TTCGCGAAGTTACGGG (SEQ ID NO: 1050)
413	(SEQ ID NO: 26)	TTTGTGAAGTTATGGGT (SEQ ID NO: 1051)
414	(SEQ ID NO: 26)	TATCGGTTCGGAGTTA (SEQ ID NO: 1052)
415	(SEQ ID NO: 26)	ATTGGTTTGGAGTTAGA (SEQ ID NO: 1053)
416	(SEQ ID NO: 26)	AAGTAGCGTCGTTATT (SEQ ID NO: 1054)
417	(SEQ ID NO: 26)	AAGTAGTGTTGTTATTGA (SEQ ID NO: 1055)
418	(SEQ ID NO: 26)	GTCGTTCGGAATTCGT (SEQ ID NO: 1056)
419	(SEQ ID NO: 26)	AGTTGTTTGGAATTTGT (SEQ ID NO: 1057)
420	(SEQ ID NO: 26)	TACGTGGTCGAGGGTT (SEQ ID NO: 1058)
421	(SEQ ID NO: 26)	TATGTGGTTGAGGGTT (SEQ ID NO: 1059)

No:	Gene	Oligo:
422	(SEQ ID NO: 26)	GGAAGTTTCGATGGTTTA (SEQ ID NO: 1060)
423	(SEQ ID NO: 26)	GGAAGTTTTGATGGTTTA (SEQ ID NO: 1061)
424	(SEQ ID NO: 38)	GGCGTTGGTATCGTTGA (SEQ ID NO: 1062)
425	(SEQ ID NO: 38)	GGTGTTGGTATTGTTGA (SEQ ID NO: 1063)
426	(SEQ ID NO: 38)	TTAAGACGCGTTTTTT (SEQ ID NO: 1064)
427	(SEQ ID NO: 38)	AAGATGTGTTTTTTGGA (SEQ ID NO: 1065)
428	(SEQ ID NO: 38)	TTTTGTCGCGGGAATT (SEQ ID NO: 1066)
429	(SEQ ID NO: 38)	TTTTTGTTGTGGGAATT (SEQ ID NO: 1067)
430	(SEQ ID NO: 38)	ATACGTAGATTCGGAG (SEQ ID NO: 1068)
431	(SEQ ID NO: 38)	TATGTAGATTTGGAGGT (SEQ ID NO: 1069)
432	(SEQ ID NO: 39)	GAAGTGGTCGTTAGTTTT (SEQ ID NO: 1070)
433	(SEQ ID NO: 39)	GAAGTGGTTGTTAGTTTTT (SEQ ID NO: 1071)
434	(SEQ ID NO: 39)	AAGGAATTCGTTTTGTAA (SEQ ID NO: 1072)
435	(SEQ ID NO: 39)	AAGGAATTTGTTTTGTAA (SEQ ID NO: 1073)
436	(SEQ ID NO: 39)	AATGTTTTCGTGATGTTG (SEQ ID NO: 1074)
437	(SEQ ID NO: 39)	AATGTTTTTGTGATGTTG (SEQ ID NO: 1075)
438	(SEQ ID NO: 39)	TAAAACGAGGGAGCGT (SEQ ID NO: 1076)
439	(SEQ ID NO: 39)	AAAATGAGGGAGTGTT (SEQ ID NO: 1077)
440	(SEQ ID NO: 27)	AGGAGTCGGTTTCGTA (SEQ ID NO: 1078)
441	(SEQ ID NO: 27)	AGGAGTTGGTTTTGTA (SEQ ID NO: 1079)
442	(SEQ ID NO: 27)	TAAAGCGCGGATATTT (SEQ ID NO: 1080)
443	(SEQ ID NO: 27)	GGGTAAAGTGTGGATA (SEQ ID NO: 1081)
444	(SEQ ID NO: 27)	TTTGAGCGGGTATCGA (SEQ ID NO: 1082)
445	(SEQ ID NO: 27)	TGAGTGGGTATTGAGT (SEQ ID NO: 1083)
446	(SEQ ID NO: 27)	TAGAGTCGAGGGGCGG (SEQ ID NO: 1084)

No:	*Gene*	*Oligo:*
447	(SEQ ID NO: 27)	TAGAGTTGAGGGGTGG (SEQ ID NO: 1085)
448	(SEQ ID NO: 27)	TTTCGAGGGACGGAAG (SEQ ID NO: 1086)
449	(SEQ ID NO: 27)	TTTTGAGGGATGGAAG (SEQ ID NO: 1087)
450	(SEQ ID NO: 27)	TATGTTTTCGGCGAAT (SEQ ID NO: 1088)
451	(SEQ ID NO: 27)	TTTTGGTGAATGGGGA (SEQ ID NO: 1089)
452	(SEQ ID NO: 27)	ATAGTCGAGGAGTCGT (SEQ ID NO: 1090)
453	(SEQ ID NO: 27)	AATAGTTGAGGAGTTGT (SEQ ID NO: 1091)
454	(SEQ ID NO: 29)	ATTTGTTTCGATTAATTT (SEQ ID NO: 1092)
455	(SEQ ID NO: 29)	ATTTGTTTTGATTAATTT (SEQ ID NO: 1093)
456	(SEQ ID NO: 29)	AATTTGCGAACGTTTGGG (SEQ ID NO: 1094)
457	(SEQ ID NO: 29)	AATTTGTGAATGTTTGGG (SEQ ID NO: 1095)
458	(SEQ ID NO: 29)	GTCGATGTTTTCGGTA (SEQ ID NO: 1096)
459	(SEQ ID NO: 29)	GGTTGATGTTTTTGGTA (SEQ ID NO: 1097)
460	(SEQ ID NO: 31)	GAGTTTCGTTATATCGT (SEQ ID NO: 1098)
461	(SEQ ID NO: 31)	GGAGTTTTGTTATATTGT (SEQ ID NO: 1099)
462	(SEQ ID NO: 31)	TTTTTGCGGTTCGATA (SEQ ID NO: 1100)
463	(SEQ ID NO: 31)	AATTTTTGTGGTTTGATA (SEQ ID NO: 1101)
464	(SEQ ID NO: 31)	TACGTTAAGGTTAACGTATA (SEQ ID NO: 1102)
465	(SEQ ID NO: 31)	TATGTTAAGGTTAATGTATA (SEQ ID NO: 1103)
466	(SEQ ID NO: 31)	TGTTTCGTCGTTATAAT (SEQ ID NO: 1104)
467	(SEQ ID NO: 31)	GTTTTGTTGTTATAATTAGA (SEQ ID NO: 1105)
468	(SEQ ID NO: 31)	GGCGTAGGTTACGATT (SEQ ID NO: 1106)
469	(SEQ ID NO: 31)	GGGGTGTAGGTTATGA (SEQ ID NO: 1107)
470	(SEQ ID NO: 31)	ATTCGTTACGGATCGT (SEQ ID NO: 1108)
471	(SEQ ID NO: 31)	TTTATTTGTTATGGATTGT (SEQ ID NO: 1109)

No:	Gene	Oligo:
472	(SEQ ID NO: 31)	AGTTTTCGGATTCGAA (SEQ ID NO: 1110)
473	(SEQ ID NO: 31)	AGAGTTTTTGGATTTGA (SEQ ID NO: 1111)
474	(SEQ ID NO: 31)	TATTTCGAGGTAGCGG (SEQ ID NO: 1112)
475	(SEQ ID NO: 31)	TTTGAGGTAGTGGGAT (SEQ ID NO: 1113)
476	(SEQ ID NO: 31)	GAGAGAAACGGTTTTTGT (SEQ ID NO: 1114)
477	(SEQ ID NO: 31)	GAGAGAAATGGTTTTTGT (SEQ ID NO: 1115)
478	(SEQ ID NO: 31)	GTTTGATGGATGTTTTT (SEQ ID NO: 1116)
479	(SEQ ID NO: 31)	GTACGACGGTAAGGAT (SEQ ID NO: 1117)
480	(SEQ ID NO: 31)	GTATGATGGTAAGGATTA (SEQ ID NO: 1118)
481	(SEQ ID NO: 31)	AGTTGTTTCGTAGATATT (SEQ ID NO: 1119)
482	(SEQ ID NO: 31)	AGTTGTTTTGTAGATATT (SEQ ID NO: 1120)
483	(SEQ ID NO: 40)	AGTAAGCGGTTGTATAT (SEQ ID NO: 1121)
484	(SEQ ID NO: 40)	AAAAGTAAGTGGTTGTAT (SEQ ID NO: 1122)
485	(SEQ ID NO: 40)	AAATTGAGCGTTTATGT (SEQ ID NO: 1123)
486	(SEQ ID NO: 40)	ATTGAGTGTTTATGTGTA (SEQ ID NO: 1124)

Table 3

Assay	left Primer	right Primer	Detection Probe
	SEQ ID NO: 16		
1	1160	1161	1162
2	1163	1164	1165
3	1166	1164	1165
4	1163	1167	1165
5	1163	1168	1165
6	1166	1167	1165
7	1166	1168	1165
8	1169	1164	1170
9	1171	1164	1170
10	1169	1167	1170

11	1169	1168	1170
12	1171	1167	1170
13	1171	1168	1170
14	1172	1164	1165
15	1173	1164	1165
16	1174	1164	1170
17	1175	1176	1177
18	1173	1167	1165
19	1174	1167	1170
20	1174	1168	1170
21	1178	1176	1177
22	1179	1180	1181
23	1179	1182	1181
24	1183	1164	1170
25	1184	1180	1181
26	1184	1182	1181
27	1183	1167	1170
28	1183	1168	1170
29	1175	1185	1177

SEQ ID NO: 47

1	1186	1187	1188
2	1186	1189	1188
3	1190	1187	1188
4	1191	1189	1188
5	1192	1189	1188
6	1191	1193	1188
7	1192	1193	1188
8	1191	1194	1188
9	1195	1193	1188
10	1195	1194	1188
11	1195	1189	1188
12	1196	1197	1188
13	1186	1198	1188
14	1186	1197	1188
15	1199	1187	1188
16	1200	1201	1202
17	1203	1193	1188
18	1203	1194	1188
19	1203	1189	1188
20	1190	1198	1188
21	1186	1204	1188
22	1190	1197	1188
23	1191	1197	1188
24	1192	1197	1188
25	1195	1197	1188
26	1205	1201	1202
27	1206	1193	1188
28	1206	1189	1188
29	1199	1198	1188
30	1199	1197	1188
31	1192	1207	1188

32	1208	1197	1188
33	1190	1204	1188
34	1209	1189	1188
35	1196	1210	1188
36	1203	1197	1188
37	1211	1212	1188
38	1213	1197	1188
39	1214	1193	1188
40	1214	1189	1188
41	1186	1215	1188
42	1216	1217	1218
43	1199	1204	1188
44	1206	1197	1188
45	1219	1197	1188
46	1190	1220	1188
47	1221	1201	1202
48	1200	1222	1202
49	1206	1207	1188
50	1223	1193	1188
51	1223	1194	1188
52	1223	1189	1188
53	1190	1224	1188
54	1205	1222	1202
55	1199	1220	1188
56	1208	1210	1188
57	1225	1226	1188
58	1214	1207	1188
59	1200	1227	1202
60	1213	1210	1188
61	1200	1228	1202
62	1199	1224	1188
63	1190	1229	1188
64	1230	1207	1188
65	1186	1231	1188
66	1219	1210	1188
67	1206	1220	1188
68	1205	1227	1202
69	1186	1232	1188
70	1223	1197	1188
71	1200	1233	1202
72	1234	1235	1236
73	1208	1237	1238
74	1186	1239	1188
75	1209	1215	1188
76	1205	1228	1202
77	1199	1229	1188
78	1240	1212	1188
79	1241	1197	1188
80	1213	1237	1238
81	1242	1243	1244
82	1206	1224	1188

83	1245	1246	1188
84	1221	1222	1202
85	1205	1233	1202
86	1247	1193	1188
87	1247	1189	1188
88	1190	1232	1188
89	1242	1248	1244
90	1249	1212	1188
91	1208	1250	1188
92	1190	1239	1188
93	1191	1251	1188
94	1192	1251	1188
95	1195	1251	1188
96	1219	1237	1188
97	1252	1235	1236
98	1190	1253	1188
99	1245	1197	1188
100	1247	1246	1188
101	1186	1254	1188
102	1213	1250	1188
103	1206	1229	1188
104	1190	1255	1188
105	1200	1256	1202
106	1199	1232	1188
107	1257	1187	1188
108	1258	1259	1188
109	1260	1261	1262
110	1221	1227	1202
111	1199	1239	1188
112	1260	1263	1262
113	1264	1235	1236
114	1219	1250	1188
115	1199	1253	1188
116	1221	1228	1202
117	1247	1197	1188
118	1265	1197	1188
119	1266	1210	1188
120	1203	1251	1188
121	1267	1268	1269
122	1186	1270	1188
123	1199	1255	1188
124	1205	1256	1202
125	1271	1272	1244
126	1273	1217	1218
127	1274	1226	1188
128	1190	1254	1188
129	1275	1276	1269
130	1277	1278	1279
131	1206	1232	1188
132	1241	1210	1188
133	1221	1233	1202

134	1280	1235	1236
135	1281	1276	1269
136	1282	1201	1202
137	1247	1207	1188
138	1234	1283	1284
139	1277	1285	1279
140	1242	1286	1262
141	1287	1276	1269
142	1288	1276	1269
143	1289	1290	1218
144	1277	1291	1279
145	1277	1292	1279
146	1206	1253	1188
147	1293	1235	1236
148	1294	1235	1236
149	1199	1254	1188
150	1257	1198	1188
151	1206	1255	1188
152	1295	1261	1262
153	1296	1220	1188
154	1271	1297	1244
155	1245	1298	1188
156	1299	1226	1188
157	1271	1300	1262
158	1301	1302	1303
159	1295	1263	1262
160	1304	1305	1244
161	1304	1243	1244
162	1245	1210	1188
163	1306	1235	1307
164	1245	1220	1188
165	1308	1243	1244
166	1225	1309	1188
167	1310	1283	1284
168	1310	1311	1284
169	1312	1235	1236
170	1242	1313	1262
171	1260	1272	1244
172	1186	1314	1188
173	1308	1248	1244
174	1252	1311	1307
175	1271	1315	1244
176	1196	1316	1188
177	1289	1217	1218
178	1247	1298	1188
179	1247	1317	1188
180	1225	1259	1188
181	1260	1318	1262
182	1257	1204	1188
183	1206	1254	1188
184	1225	1319	1188

185	1221	1256	1202
186	1320	1278	1279
187	1295	1286	1262
188	1274	1197	1188
189	1225	1237	1188
190	1321	1322	1279
191	1323	1272	1244
192	1324	1325	1326
193	1265	1210	1188
194	1301	1246	1188
195	1327	1235	1328
196	1245	1224	1188
197	1247	1220	1188
198	1329	1243	1244
199	1320	1330	1279
200	1331	1243	1244
201	1277	1332	1279
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333	1864	1862	1860
334	1828	1843	1822
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337	1871	1825	1826
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346	1870	1838	1835
347	1870	1879	1880
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349	1883	1825	1826
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352	1827	1865	1822
353	1824	1884	1826
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355	1828	1857	1822
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357	1827	1872	1822
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369	1885	1838	1835
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371	1828	1872	1822
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373	1892	1821	1822
374	1845	1893	1894
375	1895	1896	1897
376	1828	1875	1876
377	1829	1875	1876
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380	1887	1900	1889
381	1861	1837	1826
382	1824	1901	1826
383	1902	1903	1904
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386	1845	1908	1894
387	1845	1909	1910
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440	1899	1920	1889
441	1861	1905	1826
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451	1854	1865	1822
452	1874	1852	1826
453	1941	1942	1943
454	1827	1930	1876
455	1844	1872	1822
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457	1820	1945	1876
458	1864	1939	1860
459	1864	1940	1860
460	1854	1872	1822
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477	1929	1918	1889
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479	1956	1868	1869
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482	1827	1937	1876
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512	1827	1945	1876
513	1858	1973	1860
514	1911	1972	1904
515	1887	1962	1889
516	1828	1936	1822
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518	1887	1974	1889
519	1958	1855	1850
520	1958	1856	1850
521	1828	1937	1876
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523	1887	1975	1889
524	1887	1976	1889
525	1824	1977	1826
526	1845	1978	1894
527	1979	1980	1981

SEQ ID NO 32

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75	1997	1993	1991
76	1998	2007	2000
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79	2006	2004	1991
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81	1996	2010	1984

SEQ ID NO 33

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96	2074	2097	2060
97	2098	2094	2060
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4	2107	2108	2109
5	2110	2100	2101
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7	2112	2100	2103
8	2113	2100	2103
9	2114	2100	2103
10	2115	2116	2117
11	2118	2116	2117
12	2119	2100	2120
13	2121	2100	2101
14	2122	2100	2101
15	2123	2116	2117
16	2124	2125	2109
17	2126	2125	2109
18	2099	2127	2120
19	2128	2125	2109
20	2115	2129	2117
21	2118	2129	2117
22	2102	2127	2103
23	2123	2129	2117
24	2107	2130	2109
25	2099	2131	2101
26	2102	2131	2120
27	2132	2105	2106
28	2110	2127	2120
29	2133	2125	2109
30	2113	2127	2103
31	2119	2127	2103
32	2121	2127	2101
33	2122	2127	2101
34	2110	2131	2101
35	2113	2131	2120
36	2112	2131	2120
37	2114	2131	2120
38	2134	2125	2109
39	2119	2131	2101
40	2107	2135	2109
41	2121	2131	2101
42	2122	2131	2101

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44	2118	2136	2117
45	2099	2137	2101
46	2102	2137	2101
47	2099	2138	2101
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49	2102	2138	2101
50	2124	2140	2109
51	2115	2141	2117
52	2126	2140	2109
53	2099	2142	2101
54	2102	2142	2101
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68	2148	2154	2145
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70	2155	2153	2145
71	2151	2154	2145
72	2155	2154	2145
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74	2148	2150	2145
75	2149	2150	2145
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4	2157	2164	2159
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6	2157	2166	2159
7	2161	2167	2163
8	2168	2158	2159
9	2160	2164	2159
10	2160	2165	2159
11	2160	2166	2159
12	2169	2158	2159
13	2157	2170	2159
14	2160	2170	2159
15	2168	2164	2159
16	2168	2165	2159
17	2171	2164	2159

18	2171	2166	2159
19	2168	2166	2159
20	2169	2164	2159
21	2172	2164	2159
22	2169	2165	2159
23	2172	2166	2159
24	2169	2166	2159
25	2173	2174	2175
26	2161	2176	2163
27	2168	2170	2159
28	2171	2170	2159
29	2169	2170	2159
30	2172	2170	2159
31	2160	2177	2159
32	2173	2178	2175
33	2179	2162	2163
34	2161	2180	2163
35	2157	2181	2159
36	2157	2182	2159
37	2157	2183	2159
38	2160	2181	2159
39	2160	2182	2159
40	2179	2167	2163
41	2168	2177	2159
42	2169	2177	2159
43	2184	2174	2175
44	2157	2185	2159
45	2184	2178	2175
46	2186	2187	2188
47	2157	2189	2159
48	2168	2181	2159
49	2168	2182	2159
50	2157	2190	2159
51	2169	2181	2159
52	2169	2182	2159
53	2168	2183	2159
54	2191	2183	2159
55	2186	2192	2188
56	2160	2189	2159
57	2193	2194	2163
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60	2196	2187	2188
61	2171	2197	2159
62	2172	2197	2159
63	2168	2185	2159
64	2196	2192	2188
65	2179	2176	2163
66	2186	2198	2188
67	2186	2199	2188
68	2200	2194	2163

69	2168	2189	2159
70	2171	2189	2159
71	2201	2164	2159
72	2201	2166	2159
73	2169	2189	2159
74	2172	2189	2159
75	2202	2203	2204
76	2179	2180	2163
77	2168	2190	2159
78	2169	2190	2159
79	2161	2205	2163
80	2206	2162	2163
81	2196	2198	2188
82	2196	2199	2188
83	2201	2170	2159
84	2193	2162	2163
85	2207	2162	2163
86	2208	2177	2159
87	2168	2209	2159
88	2206	2167	2163
89	2169	2209	2159
90	2210	2162	2163
91	2211	2212	2213
92	2193	2167	2163
93	2207	2167	2163
94	2214	2187	2188
95	2160	2215	2159
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97	2216	2217	2159
98	2160	2218	2159
99	2210	2167	2163
100	2193	2219	2163
101	2220	2221	2222
102	2223	2203	2204
103	2224	2203	2204
104	2225	2203	2204
105	2226	2162	2163
106	2220	2227	2222
107	2168	2215	2159
108	2169	2215	2159
109	2214	2198	2188
110	2214	2199	2188
111	2228	2203	2204
112	2220	2229	2222
113	2200	2219	2163
114	2171	2218	2159
115	2168	2218	2159
116	2172	2218	2159
117	2169	2218	2159
118	2179	2205	2163
119	2230	2187	2188

120	2193	2176	2163
121	2231	2178	2175
122	2232	2174	2175
123	2206	2180	2163
124	2226	2167	2163
125	2230	2192	2188
126	2201	2189	2159
127	2207	2233	2163
128	2193	2180	2163
129	2207	2180	2163
130	2232	2178	2175
131	2234	2212	2213
132	2235	2221	2222
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134	2237	2238	2239
135	2208	2209	2159
136	2240	2203	2204
137	2241	2174	2175
138	2157	2242	2159
139	2243	2218	2159
140	2220	2244	2222
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142	2210	2180	2163
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144	2230	2198	2188
145	2230	2245	2188
146	2230	2246	2188
147	2241	2178	2175
148	2157	2247	2159
149	2157	2248	2159
150	2249	2238	2239
151	2250	2221	2222
152	2251	2162	2163
153	2160	2247	2159
154	2160	2248	2159
155	2226	2176	2163
156	2252	2221	2253
157	2254	2255	2256
158	2237	2257	2239
159	2210	2258	2163
160	2259	2221	2222
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162	2261	2221	2222
163	2262	2212	2213
164	2226	2180	2163
165	2168	2242	2159
166	2191	2242	2159
167	2261	2227	2222
168	2235	2244	2222
169	2208	2215	2159
170	2224	2212	2204

171	2251	2167	2163
172	2261	2229	2222
173	2263	2174	2175
174	2208	2218	2159
175	2264	2205	2163
176	2265	2212	2213
177	2168	2247	2159
178	2168	2248	2159
179	2169	2247	2159
180	2169	2248	2159
181	2249	2257	2239
182	2216	2247	2159
183	2266	2267	2268
184	2263	2178	2175
185	2206	2205	2163
186	2269	2255	2256
187	2266	2270	2268
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190	2273	2203	2163
191	2274	2203	2204
192	2171	2238	2159
193	2168	2238	2159
194	2193	2205	2163
195	2207	2205	2163
196	2201	2218	2159
197	2172	2238	2159
198	2169	2238	2159
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201	2276	2174	2175
202	2252	2244	2253
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SEQ ID NO 41

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63	3292	3343	3285
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15	4013	4011	4012

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254	3998	4170	4000
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262	4179	4186	4181
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403	4501	4422	4373
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414	4551	4535	4474
415	4445	4403	4392
416	4552	4396	4358
417	4390	4531	4392
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430	4408	4511	4410
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54	4754	4700	4714
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56	4726	4715	4698
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539	6543	6711	6536
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99	7672	7697	7674
100	7672	7698	7674
101	7699	7612	7668
102	7655	7678	7679
103	7656	7659	7649
104	7653	7700	7652
105	7653	7701	7679
106	7647	7684	7649
107	7655	7685	7652
108	7651	7685	7652
109	7669	7684	7649
110	7664	7657	7652
111	7663	7657	7652
112	7647	7688	7649
113	7702	7681	7682
114	7703	7695	7696
115	7704	7705	7706
116	7669	7688	7649
117	7660	7662	7649
118	7661	7662	7649
119	7672	7707	7674
120	7663	7662	7652
121	7664	7662	7652
122	7708	7659	7649

123	7647	7709	7649
124	7710	7697	7674
125	7710	7698	7674
126	7653	7711	7652
127	7683	7657	7652
128	7672	7712	7674
129	7655	7700	7652
130	7651	7700	7652
131	7651	7701	7679
132	7664	7665	7652
133	7663	7665	7652
134	7713	7648	7649
135	7714	7612	7715
136	7687	7676	7649
137	7716	7648	7717
138	7670	7662	7652
139	7663	7659	7652
140	7664	7659	7652
141	7718	7698	7719
142	7672	7720	7674
143	7658	7721	7649
144	7655	7711	7652
145	7651	7711	7652
146	7692	7676	7649
147	7683	7665	7652
148	7687	7684	7649
149	7672	7722	7674
150	7723	7697	7674
151	7723	7698	7674
152	7664	7671	7652
153	7670	7659	7652
154	7687	7688	7649
155	7680	7724	7682
156	7672	7725	7674
157	7653	7726	7679
158	7658	7727	7649
159	7692	7684	7649
160	7710	7720	7674
161	7680	7728	7682
162	7677	7662	7649
163	7658	7729	7649
164	7647	7721	7649
165	7647	7730	7649
166	7692	7688	7649
167	7669	7721	7649
168	7596	7731	7732
169	7683	7671	7652
170	7658	7733	7649
171	7647	7734	7649
172	7735	7648	7717
173	7669	7734	7649

174	7686	7671	7652
175	7736	7705	7706
176	7710	7725	7674
177	7655	7726	7679
178	7663	7678	7679
179	7737	7678	7679
180	7596	7738	7732
181	7596	7739	7732
182	7647	7740	7649
183	7741	7742	7743
184	7664	7685	7652
185	7663	7685	7652
186	7647	7744	7649
187	7702	7724	7682
188	7663	7676	7652
189	7664	7676	7652
190	7672	7745	7674
191	7672	7746	7674
192	7647	7729	7649
193	7669	7729	7649
194	7680	7747	7682
195	7723	7720	7674
196	7702	7728	7682
197	7748	7705	7706
198	7596	7749	7732
199	7750	7648	7649
200	7669	7733	7649
201	7689	7751	7691
202	7752	7698	7674
203	7752	7697	7674
204	7683	7678	7679
205	7718	7725	7719
206	7741	7753	7743
207	7647	7754	7649
208	7755	7612	7715
209	7647	7756	7649
210	7669	7754	7649
211	7683	7685	7652
212	7650	7744	7649
213	7664	7700	7652
214	7663	7700	7652
215	7663	7701	7679
216	7693	7751	7691
217	7651	7744	7652
218	7757	7697	7674
219	7757	7698	7674
220	7758	7759	7682
221	7699	7642	7715
222	7723	7725	7674
223	7710	7745	7674
224	7687	7721	7649

225	7661	7709	7649
226	7647	7760	7649
227	7672	7761	7674
228	7687	7734	7649
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231	7658	7763	7649
232	7672	7764	7674
233	7692	7721	7649
234	7683	7700	7652
235	7672	7765	7674
236	7670	7701	7679
237	7702	7747	7682
238	7664	7711	7652
239	7663	7711	7652
240	7766	7690	7767
241	7713	7662	7649
242	7692	7734	7649
243	7714	7667	7668
244	7716	7662	7717
245	7672	7768	7674
246	7769	7695	7696
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19	7793	7778	7776
20	7781	7794	7783
21	7791	7785	7779
SEQ ID NO 9			
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2	7798	7796	7797
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6	7798	7799	7797

7	7804	7796	7797
8	7800	7799	7797
9	7801	7805	7803
10	7806	7802	7803
11	7807	7802	7803
12	7798	7808	7797
13	7804	7799	7797
14	7809	7796	7797
15	7806	7805	7803
16	7810	7808	7797
17	7800	7808	7797
18	7811	7812	7813
19	7814	7802	7803
20	7807	7805	7803
21	7815	7802	7803
22	7816	7802	7813
23	7795	7817	7797
24	7809	7799	7797
25	7818	7819	7820
26	7804	7808	7797
27	7798	7821	7797
28	7822	7802	7823
29	7814	7805	7803
30	7798	7824	7797
31	7825	7819	7820
32	7826	7819	7820
33	7815	7805	7803
34	7798	7817	7797
35	7810	7821	7797
36	7800	7821	7797
37	7827	7796	7797
38	7828	7802	7803
39	7810	7824	7797
40	7800	7824	7797
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45	7837	7830	7831
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54	7845	7850	7847
55	7851	7846	7847
56	7852	7853	7854
57	7851	7850	7847

58	7855	7830	7831
59	7856	7846	7847
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61	7829	7858	7831
62	7856	7850	7847
63	7836	7857	7831
64	7836	7858	7831
65	7852	7859	7854
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67	7837	7857	7831
68	7837	7858	7831
69	7861	7853	7854
70	7862	7835	7834
71	7863	7860	7854
72	7864	7865	7866
73	7862	7838	7834
74	7867	7859	7834
75	7839	7857	7831
76	7839	7858	7831
77	7867	7838	7834
78	7861	7860	7854
79	7841	7858	7831
80	7868	7853	7834
81	7869	7859	7834
82	7870	7833	7834
83	7871	7838	7854
84	7870	7835	7834

SEQ ID NO 12

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8	7876	7879	7874
9	7880	7873	7874
10	7880	7875	7874
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12	7878	7879	7874
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14	7885	7886	7884
15	7885	7883	7884
16	7887	7873	7874
17	7887	7875	7874
18	7880	7879	7874
19	7876	7888	7874
20	7889	7873	7874
21	7889	7875	7874
22	7890	7891	7892
23	7893	7894	7895

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25	7898	7886	7884
26	7898	7883	7884
27	7899	7873	7874
28	7899	7875	7874
29	7900	7886	7884
30	7900	7883	7884
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32	7902	7873	7874
33	7902	7875	7874
34	7896	7875	7874
35	7903	7875	7874
36	7904	7905	7906
37	7878	7888	7874
38	7872	7907	7874
39	7908	7897	7874
40	7893	7909	7895
41	7889	7879	7874
42	7893	7910	7895
43	7911	7912	7913
44	7914	7891	7892
45	7915	7873	7874
46	7908	7875	7874
47	7887	7877	7874
48	7916	7905	7906
49	7915	7917	7874
50	7918	7891	7892
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52	7878	7901	7874
53	7880	7888	7874
54	7899	7879	7874
55	7915	7921	7874
56	7922	7912	7913
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58	7924	7920	7906
59	7925	7873	7874
60	7925	7875	7874
61	7926	7894	7895
62	7927	7873	7874
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65	7919	7905	7906
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69	7899	7877	7874
70	7933	7920	7895
71	7934	7920	7931
72	7902	7877	7874
73	7896	7877	7874
74	7924	7905	7906

75	7880	7901	7874
76	7928	7905	7895
77	7887	7881	7874
78	7930	7905	7931
79	7929	7935	7874
80	7932	7905	7906
81	7904	7936	7906
82	7933	7905	7895
83	7934	7905	7931
84	7898	7923	7884
85	7893	7937	7895
86	7896	7935	7874
87	7902	7935	7874
88	7926	7909	7895
89	7900	7923	7884
90	7938	7939	7884
91	7889	7888	7874
92	7926	7910	7895
93	7940	7941	7942
94	7940	7943	7942
95	7908	7877	7874
96	7902	7944	7874
97	7896	7944	7874
98	7903	7944	7874
99	7945	7946	7947
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SEQ ID NO 20

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7	7949	7959	7960
8	7949	7961	7957
9	7962	7950	7951
10	7963	7964	7965
11	7966	7967	7968
12	7949	7969	7951
13	7970	7967	7968
14	7949	7971	7951
15	7949	7972	7951
16	7949	7973	7957
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18	7976	7977	7978
19	7966	7979	7968
20	7952	7980	7954
21	7952	7981	7954
22	7970	7979	7968
23	7974	7982	7975
24	7983	7984	7960

25	7962	7956	7957
26	7985	7967	7968
27	7976	7986	7978
28	7987	7964	7965
29	7949	7988	7989
30	7958	7980	7954
31	7958	7981	7954
32	7949	7990	7989
33	7949	7991	7951
34	7962	7984	7960
35	7985	7979	7968
36	7992	7964	7993
37	7994	7995	7968
38	7996	7995	7968
39	7997	7956	7957
40	7998	7999	7957
41	7962	7969	7951
42	7963	8000	7965
43	7974	8001	7975
44	8002	8003	7954
45	7966	7995	7968
46	8004	7995	7968
47	7997	7984	7960
48	7970	7995	7968
49	7962	7973	7957
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51	8006	7995	7968
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54	7974	8008	7975
55	8009	7977	7978
56	8010	8003	7954
57	8011	8012	8013
58	8002	8014	7954
59	8015	8016	8013
60	7949	8017	7989
61	8018	8019	8013
62	8020	8016	8013
63	8009	7986	7978
64	7987	8000	7965
65	7998	8021	7957
66	7983	8022	7957
67	8023	8024	7954
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69	7962	7988	7989
70	7985	7995	7968
71	7983	8026	7957
72	7963	8027	7965
73	7974	8028	7975
74	8029	8030	7978
75	7997	7973	7957

76	7962	7990	7989
77	8031	8012	8013
78	8018	8032	8013
79	8002	8033	7954
80	7962	7991	7951
81	8010	8014	7954
82	8002	8034	7954
83	7949	8035	8036
84	8037	7959	7960
85	8037	7961	7957
86	8015	8012	8013
87	8038	8012	8013
88	7974	8039	7975
89	7974	8040	7975
90	7949	8041	8036
91	7998	8042	7960
92	8043	8044	8045
93	8046	8047	8048
94	8049	8024	7954
95	7962	8050	7951
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124	8080	8076	8081
125	8082	8083	8084
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127	8086	8061	8087
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129	8088	8070	8079
130	8054	8085	8053
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133	8093	8061	8094
134	8095	8072	8073
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136	8096	8076	8097
137	8075	8070	8077
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140	8086	8074	8087
141	8080	8068	8081
142	8080	8070	8081
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146	8103	8104	8053
147	8051	8105	8053
148	8051	8106	8053
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150	8051	8107	8053
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155	8112	8070	8113
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159	8114	8117	8116
160	8067	8118	8069
161	8119	8061	8062
162	8099	8074	8094
163	8096	8068	8097
164	8120	8068	8121
165	8096	8070	8097
166	8086	8122	8087
167	8123	8104	8053
168	8078	8118	8079
169	8114	8124	8116
170	8071	8125	8073
171	8090	8126	8092
172	8127	8061	8062
173	8051	8128	8053
174	8071	8129	8073
175	8071	8130	8073
176	8131	8061	8087
177	8103	8098	8053

178	8123	8085	8053
179	8054	8128	8053
180	8132	8133	8062
181	8119	8074	8062
182	8088	8118	8079
183	8111	8068	8097
184	8111	8070	8097
185	8134	8072	8073
186	8082	8135	8101
187	8103	8105	8053
188	8136	8083	8084
189	8137	8083	8084
190	8138	8122	8062
191	8139	8115	8116
192	8139	8117	8116
193	8114	8109	8116
194	8140	8070	8113
195	8127	8074	8062
196	8141	8115	8116
197	8141	8117	8116
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199	8142	8102	8084
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203	8131	8074	8087
204	8082	8146	8147
205	8095	8125	8073
206	8123	8098	8053
207	8139	8124	8116
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209	8149	8115	8116
210	8149	8117	8116
211	8150	8151	8152
212	8078	8144	8145
213	8071	8153	8073
214	8095	8130	8073
215	8095	8129	8073
216	8119	8122	8062
217	8141	8124	8116
218	8154	8058	8053
219	8114	8155	8116
220	8103	8156	8053
221	8123	8105	8053
222	8149	8124	8116
223	8157	8061	8062
224	8158	8061	8094
225	8159	8160	8161
226	8162	8163	8164
227	8165	8061	8087
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232	8136	8102	8101
233	8112	8118	8113
234	8169	8109	8110
235	8170	8061	8094
236	8127	8122	8062
237	8088	8144	8145
238	8131	8122	8087
239	8171	8133	8062
240	8172	8061	8094
241	8139	8109	8116
242	8173	8068	8121
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244	8090	8175	8092
245	8176	8125	8073
SEQ ID NO 35			
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7	8188	8181	8179
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9	8192	8190	8191
10	8193	8190	8191
11	8182	8194	8184
12	8195	8190	8191
13	8196	8183	8184
14	8185	8194	8184
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17	8187	8186	8179
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21	8198	8203	8200
22	8204	8205	8200
23	8206	8183	8184
24	8192	8201	8191
25	8192	8202	8191
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28	8189	8207	8191
29	8192	8207	8191
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31	8208	8194	8184
32	8196	8194	8184
33	8209	8202	8191

34	8195	8201	8191
35	8195	8202	8191
36	8182	8210	8184
37	8198	8211	8200
38	8198	8212	8200
39	8213	8214	8215
40	8213	8216	8215
41	8213	8217	8215
42	8218	8178	8179
43	8195	8207	8191
44	8219	8202	8191
45	8198	8220	8200
46	8213	8221	8215
47	8222	8178	8179
48	8223	8178	8179
49	8185	8210	8184
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51	8227	8181	8228
52	8229	8230	8231
53	8177	8232	8179
54	8233	8234	8235
55	8206	8194	8184
56	8236	8199	8200
57	8237	8194	8184
58	8187	8238	8179
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61	8240	8214	8215
62	8240	8216	8215
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64	8182	8241	8184
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71	8252	8253	8254
72	8198	8255	8200
73	8236	8205	8200
74	8236	8203	8200
75	8256	8257	8258
76	8259	8199	8200
77	8189	8260	8191
78	8224	8261	8226
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82	8262	8183	8184
83	8209	8263	8251
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88	8264	8267	8266
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91	8270	8271	8272
92	8273	8265	8266
93	8273	8267	8266
94	8274	8265	8266
95	8274	8267	8266
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100	8273	8282	8266
101	8283	8265	8266
102	8283	8267	8266
103	8279	8284	8281
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112	8286	8285	8281
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114	8294	8290	8277
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116	8270	8296	8272
117	8264	8297	8266
118	8268	8297	8266
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123	8301	8292	8277
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132	8264	8309	8266
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138	8279	8313	8281
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140	8275	8315	8277
141	8264	8316	8266
142	8307	8282	8266
143	8275	8317	8277
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147	8294	8317	8277
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152	8269	8316	8266
153	8321	8271	8272
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160	8273	8297	8266
161	8291	8280	8277
162	8325	8284	8281
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166	8273	8326	8266
167	8274	8297	8266
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172	8264	8329	8266
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177	8322	8282	8266
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182	8324	8305	8289
183	8278	8326	8266
184	8270	8332	8272
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227	8347	8267	8348
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236	8283	8309	8266
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267	8294	8370	8371
268	8295	8370	8371
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